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A Study of the Borate-Carbohydrate Complex Formed in an Aqueous Medium

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A STUDY OF THE BORATE-CARBOHYDRATE COMPLEX FORMED IN AN AQUEOUS MEDIUM

A thesis submitted by

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SUMMARY

The formation of a complex between the borate anion and the carbohydrate molecule is well established, but the stability of these complexes has received little attention. In the present study, the equilibrium concentrations of the components of the borate-carbohydrate system were determined as a function of the ratio of the initial concentrations of carbohydrate to borate. The simultaneous equations method was used in conjunction with refractive index and optical rotation measurements to determine the equilibrium concentrations. The stability constants for the several equilibria were calculated directly and served as a measure of complexing ability. Polyborate anion formation was avoided by careful control of experimental conditions.

Four model compounds were used: methyl-α-D-galactopyranoside, methyl-α-D-mannopyranoside, methyl-4-O-methyl-α-D-mannopyranoside, and methyl-4-O-methyl-β-D-mannopyranoside. These compounds are specifically related to the guar gum polymer galactomannan, but also serve for a general interpretation of the borate complex. The methyl-4-O-methyl-β-D-mannopyranoside represented a new compound and was prepared by direct methylation of 4-O-methyl-D-mannose with dimethyl sulfate. This glycoside resisted all attempts to prepare the crystalline product and was obtained as a chromatographically pure sirup. After tentative identification by paper chromatography, the compound proved to be nonreducing and on acid hydrolysis gave only 4-O-methyl-D-mannose. A specific optical rotation value of -58.3° in water was obtained, which checked closely with the value predicted by Hudson's isorotation rules. The concentration of the β-glycoside was determined spectrophotometrically, using acidic orcinol as the color reagent and the α-glycoside of 4-O-methyl-D-mannose as the reference compound.

Two types of borate complexes were found: the monocomplex, composed of one molecule of carbohydrate and one borate anion, and the dicomplex, composed of

two molecules of carbohydrate bound together by one borate anion. The 4-O-methyl-α-mannoside gave the most total complexing, forming large amounts of both the monocomplex and the dicomplex. The α-galactoside and the α-mannoside formed less of the dicomplex, but due to increased amounts of the monocomplex, formed only slightly less total complex. In contrast, the 4-O-methyl-β-mannoside gave predominantly the dicomplex, with only small amounts of the monocomplex. It also formed the least total amount of complex of the model compounds.

In the conditions employed in this study, the borate complex formed preferentially with the adjacent cis-hydroxyl grouping, giving two types of borate complex, i.e., the monocomplex and the dicomplex. The predominant type of complex present at equilibrium was controlled by adjustment of the initial ratio of concentrations of the glycoside and the borate anion. The stability of the complexes was related both to hydrogen bonding and steric hindrance factors. The stability of the monocomplex was directly related to increasing hydrogen bonding opportunities between the glycoside molecule and the borate hydroxyls, while steric hindrance explained the relative stabilities of the dicomplexes.

Upon extending the results obtained with the glycosides to the galactomannan polymer, it was found that monocomplex formation is most likely on the galactose side chain. The dicomplex was of equal likelihood on the mannose backbone or the galactose side chain. This offers an explanation for the poor solubility of straight-chain mannans in borate solutions in comparison to the galactomannans, as the monocomplex will increase water solubility.

INTRODUCTION

It has been known for many years that the addition of sodium borate to an aqueous solution of guar gum, locust bean gum, or other galactomannan-containing mucilages results in gel formation (1-4). The effect of various experimental conditions on the formation of the gel or, in cases where the polymer concentration was low, on the increase in viscosity of the solution has received considerable attention. For example, several workers (5-7) have reported that the viscosity of a galactomannan increased to a maximum and then decreased as borax was added. Other factors such as temperature, pH, and the presence of polyhydric materials or salts also have been noted to affect the viscosity of a galactomannan-borate solution (1-10).

It is generally agreed that the cis-hydroxyl groups on the mannose unit of the galactomannan polymer are the most likely point of complex formation with the borate, but the exact composition of the complex and the effect of experimental conditions and polymer structure on the composition are not known (3, 4, 6, 9, 10).

Borate solutions are also important in the extraction and solvation of several hemicelluloses (11, 12). An explanation of the borate-carbohydrate system should also account for the action of borate solution as hemicellulose solvents, in addition to the gelation effect on galactomannans.

The present study of the borate complex furnished suitable stability constants for several model compounds, and placed the interpretation of borate-carbohydrate system on a quantitative basis, as opposed to the largely qualitative nature of the previous results. While the model compounds were specifically

related to the galactomannan polymer, the results were of value in a general interpretation of the borate-carbohydrate system. Before entering into a general discussion of the borate-carbohydrate complex, the boric acid-water system must be reviewed.

THE BORIC ACID-WATER SYSTEM

In a brief summary of current opinion on aqueous solutions of boric acid, Dale (13) reviewed two points which are of prime importance in this study.

1. In an aqueous solution, boric acid acts only as a monobasic, Lewis acid. It does not function as a proton donor, but rather as an electron acceptor, giving the tetrahedral borate anion. The ionization of boric acid is given in the following equation.

$$B(OH)_3 + 2H_2O \rightleftharpoons B(OH)_4^- + H_3O^+ \qquad (1).$$

The equilibrium constant for the above reaction was determined by several workers (14-16) and found to be approximately 10^{-9} mole/liter. Using this value for the equilibrium constant, it can be shown that above a pH value of 11.0 all of the boric acid is converted into its ionic components.

2. In dilute alkaline solutions of boric acid, few if any polyions exist. This point has been investigated extensively by Ingri and co-workers (15, 16), who used potentiometric titrations of boric acid to study the condensation of the trigonal boric acid molecule with the tetrahedral borate anion. An assortment of polyborate anions was found; however, at pH levels above approximately 10.0, only the tetrahedral monovalent anion existed. Edwards and co-workers (17), from an analysis of the Raman spectra of potassium borate solutions, also concluded that the tetrahedral anion was the only component that existed in dilute alkaline solutions.

Therefore, as long as the hydrogen ion concentration is kept low, e.g., pH values of 11.0 and above, it can be assumed that the trigonal boric acid molecule is completely converted to the tetrahedral $B(OH)_4^-$ anion.

THE BORATE-POLYHYDROXYL COMPLEX

CYCLIC BORON COMPOUNDS

The structure of heterocyclic organic boron compounds was discussed in detail by Maitlis (18). Trigonal boron in the ground state has two 2s and one 2p electrons available for bond formation. Hence, trivalent boron compounds such as boric acid do not have a complete outer shell of valence electrons and can be considered electron deficient. The boron atom is therefore very reactive to any group which can donate electrons and thereby stabilize the boron atom. This accounts for the action of trigonal boric acid as a Lewis acid, accepting an electron-pair from the oxygen atom of a water molecule to give the tetrahedral $B(OH)_4^-$ anion.

In some cases, such as with borate esters, internal stabilization is affected by "back co-ordination" of the lone-pair electrons of oxygen. In these cases, structures such as $>\overset{\ominus}{B}=\overset{\oplus}{O}-$ become important. One would predict that the addition of an alkyl group would favor electron-release and would increase the double-bond character of the boron-oxygen bond. However, the infrared studies of Aubrey, Lappert, and Pysora (19) showed the opposite effect. The authors attributed this to increased steric strain, with subsequent bond lengthening. This agrees with Maitlis (18), who noted that attempts to form five-membered ester rings with cyclic diols and borate failed due to distortion of bond angles which decreased the stabilizing $-O=B<$ back co-ordination. The work of Dale (13) showed that the 5-membered ring formed with diols was stable only if the boron shifted to tetrahedral valency by the addition of a hydroxyl group.

The ring strain obtained in a 5-membered ester ring, therefore, increases the tendency to add hydroxyl ions. This accords with the general observation of many workers such as Antikainen (20) that the compound formed between boric acid and a polyol is a stronger acid than boric acid itself. This general picture of the boron atom supports the postulate that the tetrahedral form of the boron atom is present in the borate-carbohydrate complex formed in an alkaline medium.

THE BORATE-CARBOHYDRATE COMPLEX

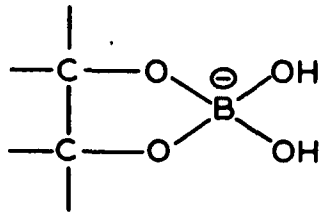
The methods used to study the borate complex have been based on the following factors: the ionization of boric acid induced by complex formation, with the resulting changes in conductivity and pH; the ionic character of the complex which leads to electrophoretic mobility and ion exchange properties; and the formation of a new structure, giving changes in optical rotation, nuclear magnetic resonance, and similar properties. The majority of the studies have been concerned not only with the composition of the complex, but also with the related equilibria.

Composition of the Borate Complexes

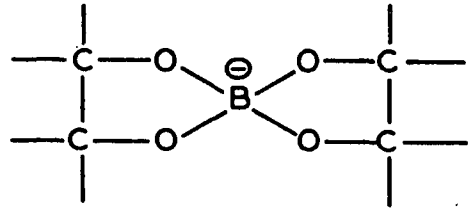
Early investigations of the borate-polyol complex followed complex formation by conductivity and pH measurements. Böeseken (21) made extensive use of these methods and laid the basic groundwork for the present-day knowledge of the borate complex. It was found that while no complex formed unless adjacent hydroxyl groups were present, this grouping by itself was not a sufficient condition for complex formation. It was also necessary for the hydroxyl groups to have restricted rotation before a complex formed. For example, a simple glycol molecule which has relatively free rotation of hydroxyl caused only a slight

conductivity increase, while a more complex molecule such as mannitol caused a considerable increase. Later work (14, 20, 22-26) with mannitol, sorbitol, and other polyols, using conductivity and pH measurements, confirmed this.

The results of these experiments indicated two types of borate-polyol complexes were formed. In the monocomplex, the borate and polyol combined in a 1:1 ratio, while in the dicomplex the borate anion combined with two polyol molecules. The structures of the two complexes, which will be referred to as BD and BD_2, respectively (see glossary), follow:

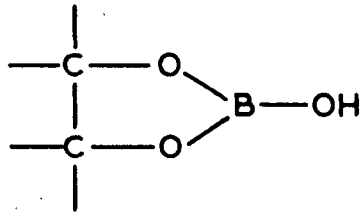


(BD)



(BD)

Under acidic conditions, the borate ester, denoted as E, was also indicated with the following structure.



(E)

Antikainen (20) also found evidence of a complex formed between two molecules of borate and one molecule of mannitol.

Mehta and Kantak (26) investigated pH changes with many common sugars and found it was necessary to assume the BD and BD_2 complex to explain their results. It was also noted that the aldehydic or ketonic group in the sugar molecule

augmented the ionization of boric acid. This points up the difficulties encountered in interpreting the pH results of complex formation with free sugars. Not only are the pH values such that complications arise due to the presence of polyborate anions, but there are also the complications due to equilibrium that exists between the furanose, pyranose, and straight-chain aldo forms of the free sugar.

Edwards and co-workers (27, 28), working with both boric acid and monosubstituted borate esters, used pH measurements to calculate the stability constants for several complexes, including those of mannose and galactose. The results were consistent with the view that the complex forms only with the borate anion, giving the BD and BD_2 complexes. With mannitol and sorbitol, the presence of the diborate complex, B_2D, prevented an evaluation of stability constants.

Upon extending the work on polyols to cover the common pentose and hexose sugars where the ring structure greatly restricts motion, Böeseken (21) found that sugars containing cis-hydroxyl groups gave a greater increase in conductivity than those containing only the trans configuration. These findings were supported by Foster and co-workers (29-34), who followed ionophoretic migration in alkaline borate electrolytes. Compounds with adjacent cis-hydroxyl groups moved rapidly during ionophoresis, while those with the trans grouping moved slowly, if at all. For example, 2,4-di-O-methyl-L-rhamnose migrated only slightly, while 3,4-di-O-methyl-L-rhamnose migrated quite rapidly (35). Only the latter compound possesses cis-hydroxyl groups.

When glycosides are used instead of free sugars, the ring structure is fixed, and the interpretation of results is greatly simplified. It has been shown that adjacent trans-hydroxyl groups do not react with the borate anion. No movement

was obtained with the methyl-D-xylopyranosides, while the methyl-D-arabinopyranosides which contain cis-hydroxyls migrated readily (34). The similar movement of methyl-D-galactopyranosides and methyl-D-arabinopyranosides (35) indicated their reactions were independent of the configuration at the glycosidic center. This was expected as the complex forms across the C_3-C_4 cis-hydroxyl groups in both cases and is removed from the glycosidic center.

In cases where the glycosidic center is adjacent to the point of complex formation, the anomeric configuration of the glycoside affects the formation of the complex. For example, the glycosidic methyl group did not hinder the 2,3-complex in methyl-α-D-mannopyranoside as it did in the β-mannoside where the glycosidic group is cis-related to the point of complex formation, and the α-mannoside had a higher ionophoretic movement than did the β-mannoside (35).

Bouveng and Lindberg (36) investigated several disaccharides by ionophoresis and found similar results to the monosaccharides. For example, 3-β-galactopyranosyl galactopyranose had a slower migration rate than did the 6-β-disaccharide, as a result of the blocked C_3-C_4 hydroxyls.

Some ionophoretic migration occurred that suggested complexing occurred across other than adjacent hydroxyl groups. For example, 2,3-di-O-methyl-D-glucose which has no adjacent cis-hydroxyl groups showed some movement (30). When interpreting these results, it must be remembered that both the cyclic and straight-chain forms are possible, with the resulting complications. The possibility of a complex forming across the C_4-C_6 hydroxyl groups was given support by the movement of the methyl-D-glucopyranosides (30) during ionophoresis. However, the relatively low electrophoretic migration indicated a weak complex.

Several workers in other areas have obtained supporting evidence for the BD or BD_2 complex and for their formation across adjacent cis-hydroxyl groups. Isbell and co-workers (37) have made an extensive study of the effect of complex formation on the optical activity of several polyols and sugars. Evidence was obtained for the BD and BD_2 complexes, with the ester E obtained in some cases. Although no quantitative data concerning the actual concentration of the complexes was obtained, the specific optical rotation of many of the complexes was estimated.

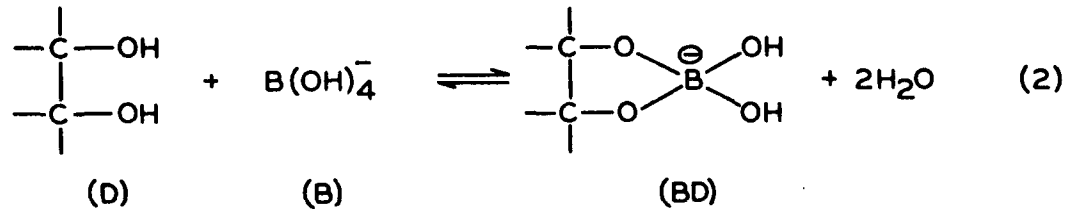
Lock and Richards (38), in a study of the absorption of borate complexes on anionic resins, noted that methyl-α-D-mannopyranoside, which has the cis-grouping, complexed readily, while methyl-α-D-glucopyranoside with no cis-hydroxyl groups complexed only slightly and was easily removed.

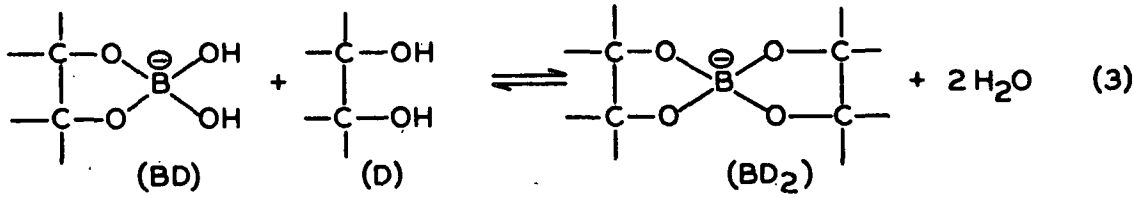
Lenz and Heeschen (39) used NMR to study the glucose-borate complex and found that only the α-anomer formed a complex, with the complex occurring between the C_1-C_2 cis-hydroxyl groups. Taken together, the experimental evidence indicates quite clearly that the BD or BD_2 complex forms preferably across adjacent cis-hydroxyl groups.

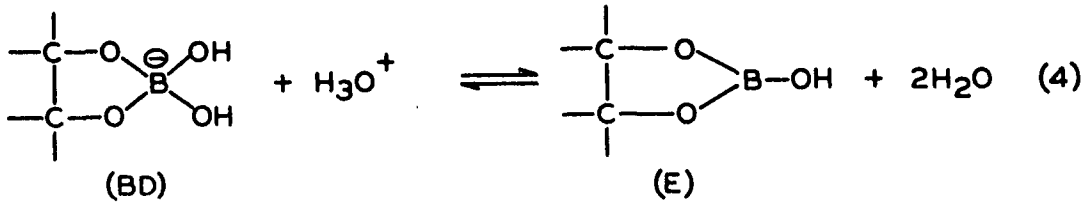
Although none of the borate complexes of carbohydrates have been separated, several complexes of similar compounds have been isolated as crystalline products. For example, Dale (13) prepared the BD complex of cis-cyclopentane-1,2-diol and cis-cyclohexane-1,2-diol. Pentaerythritol gave a complex which was probably polymeric and had the BD_2 structure. These serve as illustrative examples of the many other similar complexes which have been obtained.

Equilibria in the Borate System

The following equilibria among the borate complexes were postulated by Böeseken (21).







These reactions also serve to define the symbols D and B, which will be used throughout this discussion.

In alkaline conditions, Reaction (4) will be far to the left and can be ignored, leaving the formation of BD and BD_2 as the reactions of importance when the borate complex is formed in an alkaline solution.

In agreement with this, Isbell, et al. (37), from a series of optical rotation studies, noted that in acidic solutions the equilibria shift toward the trigonal boric acid, and only small amounts of the tetrahedral borate anion are present. Hence, there is little tendency for the BD and BD_2 complexes to form. However, in alkaline solutions the reverse is true, and there is a much greater

tendency for complex formation to occur. Only under alkaline conditions was enough complex formed to give readily noticeable changes in optical rotation.

The dependence of complex formation on the pH of the solution has also been reported by several other workers (40, 41). It was found that changes in optical activity of sugar solutions caused by borax additions could be controlled by adjustment of the pH of the solution. These changes in optical rotation were due to shifts in the boric acid equilibrium and were not a result of alkaline degradation or changes in the hydronium ion concentration. The necessity of alkaline conditions for strong complex formation has also been confirmed by NMR studies (39, 42) and ionophoretic mobility investigations (43).

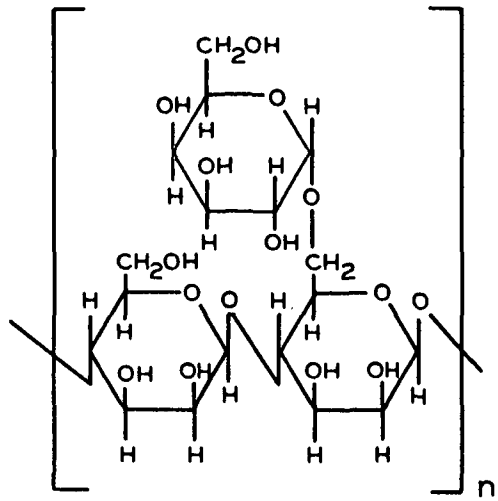
From a general consideration of stability constants, it can be shown that the BD complex is favored by high borate concentrations and by dilute solutions, while the BD_2 complex is favored by low borate concentrations and high carbohydrate concentrations. As a result, the type and relative concentration of the borate complexes can be controlled by suitable adjustment of experimental conditions. For example, Böeseken (23) followed the optical rotation while changing the ratio of mannitol to sodium borate and was able to estimate the specific optical rotations of the BD and BD_2 complexes. Isbell, et al. (37) worked with several sugars and found that the borate-to-carbohydrate ratio determined the specific optical rotation, while Haug (9) noted similar effects with several glycosides. Zill and co-workers (44-46) separated polyhydric compounds by elution of anion exchange columns with borate solutions and reported that the rate and order of elution was controlled by the pH and borate concentration of the developer. These results serve as examples of the large effect of experimental conditions on the borate complex.

In summary, most of the information on the borate complex is of a qualitative nature. Few attempts have been made to actually determine the concentration of the complexes, and these determinations have not taken into account the possible effects of polyborate anions. As far as the galactomannan polymer is concerned, there are no quantitative data available, neither in regard to the actual polymer nor for related model compounds.

MODEL COMPOUND PRODUCTION

SELECTION OF MODEL COMPOUNDS

The model compounds used in the study were selected to represent guar gum, which has the following general structure.



Methyl-α-D-galactopyranoside was chosen to represent the galactose side chain, and methyl-4-O-methyl-β-D-mannopyranoside the mannose backbone of the polymer. In order to determine the effect of side groups and different positions of complex formation, methyl-α-D-mannopyranoside and methyl-4-O-methyl-α-D-mannopyranoside were included in the study. The structures of these four model compounds are given in Fig. 1.

As previously stated, the borate complex forms across adjacent cis-hydroxyls. Therefore, the borate complex forms on the C_3-C_4 hydroxyls in the α-galactoside and on the C_2-C_3 hydroxyls of the α-mannoside, and a comparison of these two shows the effect of different positioning on complex formation. The α-mannoside and the 4-O-methyl-α-mannoside not only show the effect of an adjacent methyl group, but because there is only one pair of adjacent cis-hydroxyls in the substituted glycoside, also indicate if there is significant complexing on other than

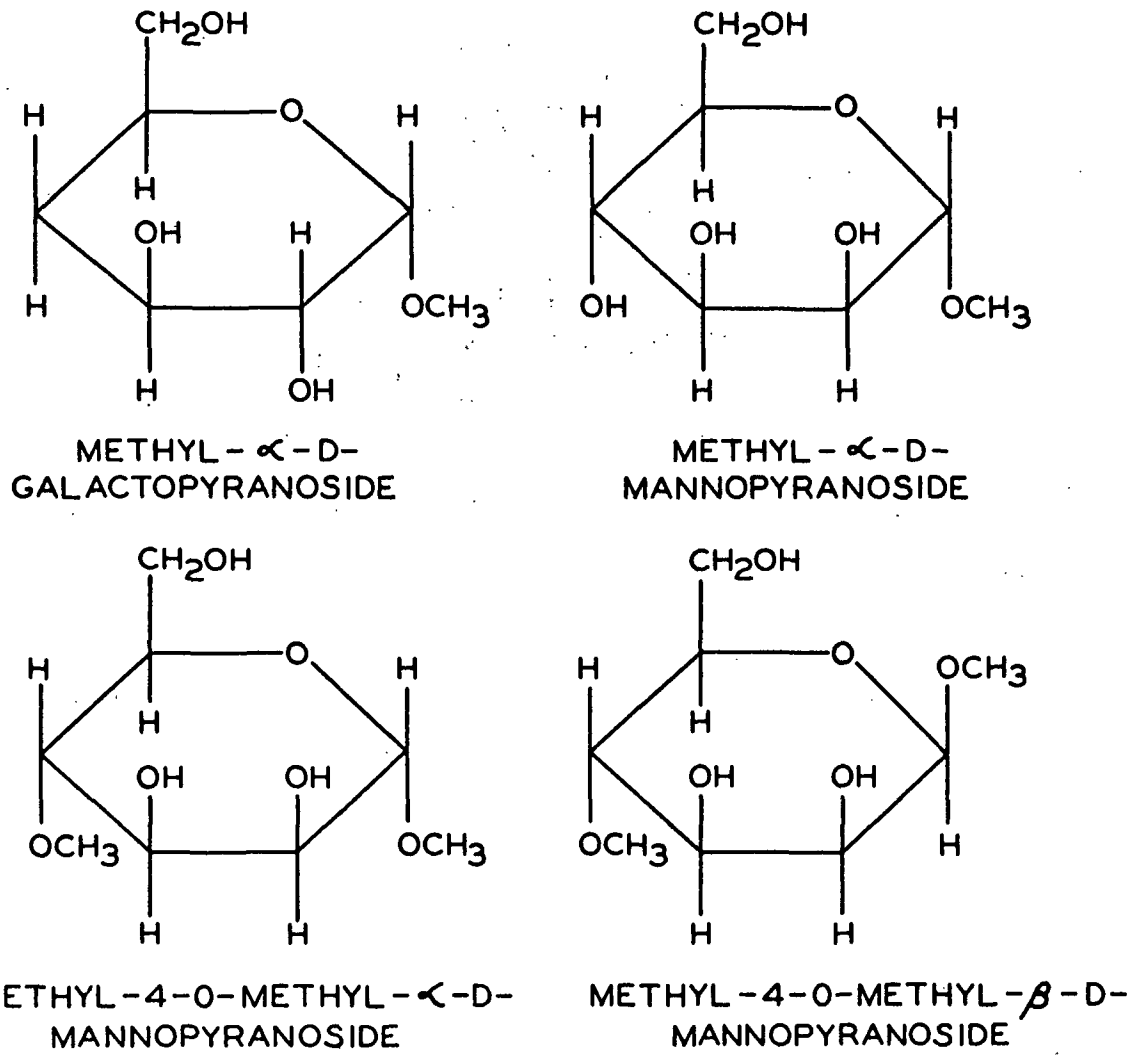
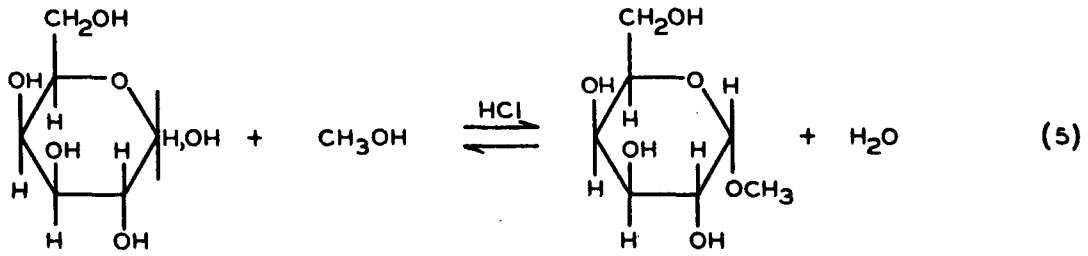


Figure 1. Structure of Model Compounds Used in This Study

cis-hydroxyls. A comparison of the α- and β-glycosides of 4-O-methyl-D-mannose shows the results of the anomeric change in configuration at a point adjacent to the position of complex formation. The results with the α-galactoside and the 4-O-methyl-β-mannoside are related directly to the galactomannan polymer.

PREPARATION OF METHYL-α-D-GALACTOPYRANOSIDE

The α-galactoside was prepared by refluxing in methanol with HCl as a catalyst. The reaction is given in Equation (5).



(5)

D-GALACTOSE METHYL-α-D-GALACTOPYRANOSIDE

The method of Dale and Hudson was followed (47). The experimental details are given in Appendix I. The total amount of anhydrous α-galactoside product obtained was 12.7 g. The physical properties were as follows:

1. Melting point of the monohydrate, 113-115°C. (literature, 111-112°C.) (48).
2. Specific rotation of anhydrous compound, +195.5° in water at 589 mμ (literature value, 196.2°) (47).

PREPARATION OF THE 4-O-METHYL-D-MANNOSE

Before the glycosides of 4-O-methyl-D-mannose could be prepared, it was necessary to produce the free sugar. The path taken to produce the free sugar is illustrated in Fig. 2. The procedures leading to the production of

4-O-methyl-D-mannose were essentially those reported in the literature, and the experimental details are given in Appendix I.

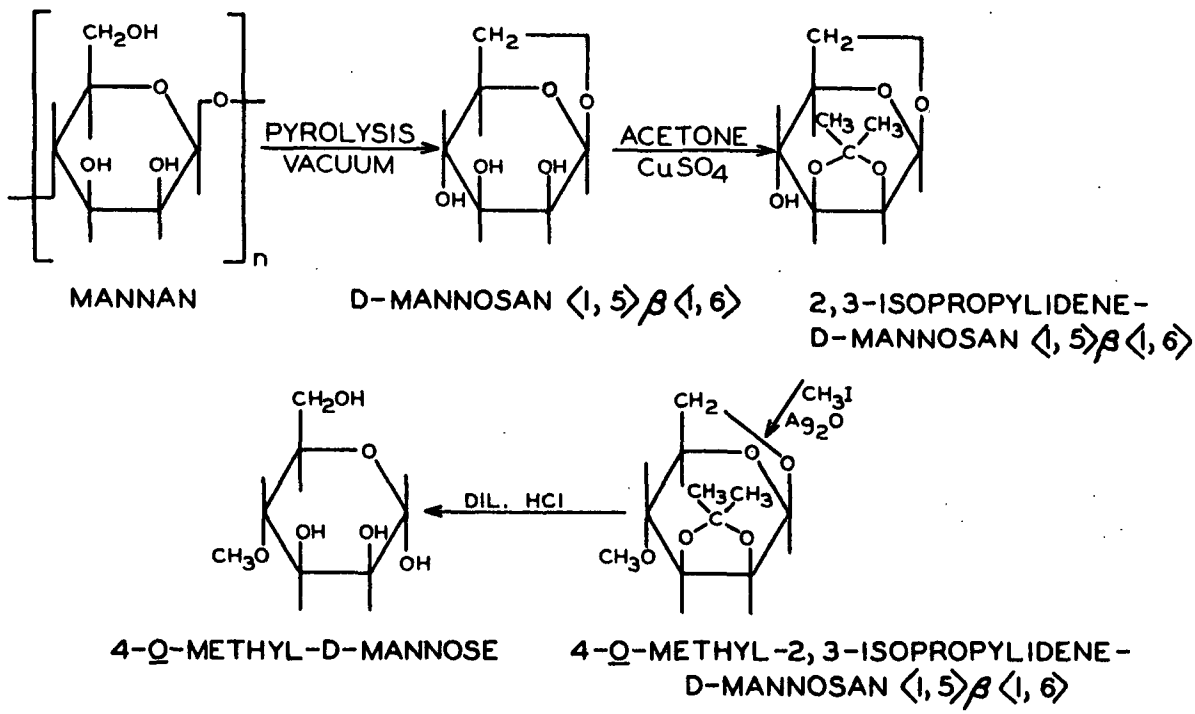


Figure 2. Preparation of 4-O-methyl-D-mannose

The crystalline 4-O-methyl-D-mannose was analyzed chromatographically and was found to contain only small traces of mannose. Because the yield of 4-O-methyl-D-mannose was lower than expected, no further purification was tried. The physical values follow:

1. Melting point, 128-130°C. (literature, 127-128°C.) (49).
2. Specific rotation, 24.72° in water at 589 mμ (literature, 22.6°) (49).

PREPARATION OF THE METHYL GLYCOSIDES OF 4-O-METHYL-D-MANNOSE

The last step in the preparation of the mannose model compounds was the formation of the glycosides of 4-O-methyl-D-mannose. The usual procedures follow

either the Fischer method (CH_3OH-HCl) or the Koenigs-Knorr reaction. However, the Fischer method yields mainly the α-glycoside when used on mannose, with only negligible amounts of the β-anomer occurring (50), and the Koenigs-Knorr reaction fails with mannose, giving an orthoester in place of the desired glycoside (51). Isbell and Frush (52) have reported that direct alkylation of mannose with one equivalent of dimethyl sulfate at 0°C. gave preferential alkylation of the glycosidic hydroxyl. Although the α-glycoside still predominated, enough of the β-form was present to permit its isolation.

Because the yield of the β-glycoside of 4-O-methyl-D-mannose was expected to be small, it was necessary to recover the unreacted sugar for recycling. For the recovery process, it is essential that only the glycosidic hydroxyl group reacts, because reaction of nonglycosidic hydroxyl groups will lead to a permanent loss of 4-O-methyl-D-mannose. Therefore, before the glycosides of 4-O-methyl-D-mannose were formed, an investigation was made of the reaction products. Unsubstituted mannose was used for the investigation as the amount of available 4-O-methyl-D-mannose was limited.

PREPARATION OF THE METHYL GLYCOSIDES OF MANNOSE BY DIRECT ALKYLATION

Direct alkylation of mannose not only served to check the procedure before use on the limited amount of 4-O-methyl-D-mannose, but also produced the second model compound, methyl-α-D-mannopyranoside. The procedure of Isbell and Frush (52) for the production of the methyl glycosides is outlined in Fig. 3. The experimental details are given in Appendix I.

Of main interest was the amount of methylation that occurred on the nonglycosidic hydroxyl groups. At all stages, paper chromatography was used to determine if excessive direct methylation was occurring. During several runs,

the temperature and pH during the methylation stage were changed to see what tolerances were allowable before the unwanted methylation occurred. It was found that as long as the temperature was near 0°C. (± 4-5°C.) and the pH was in the 10.5-11.5 range, no major amounts of nonglycosidic methylation occurred. The physical properties of the compounds produced in the study are given in Table I.

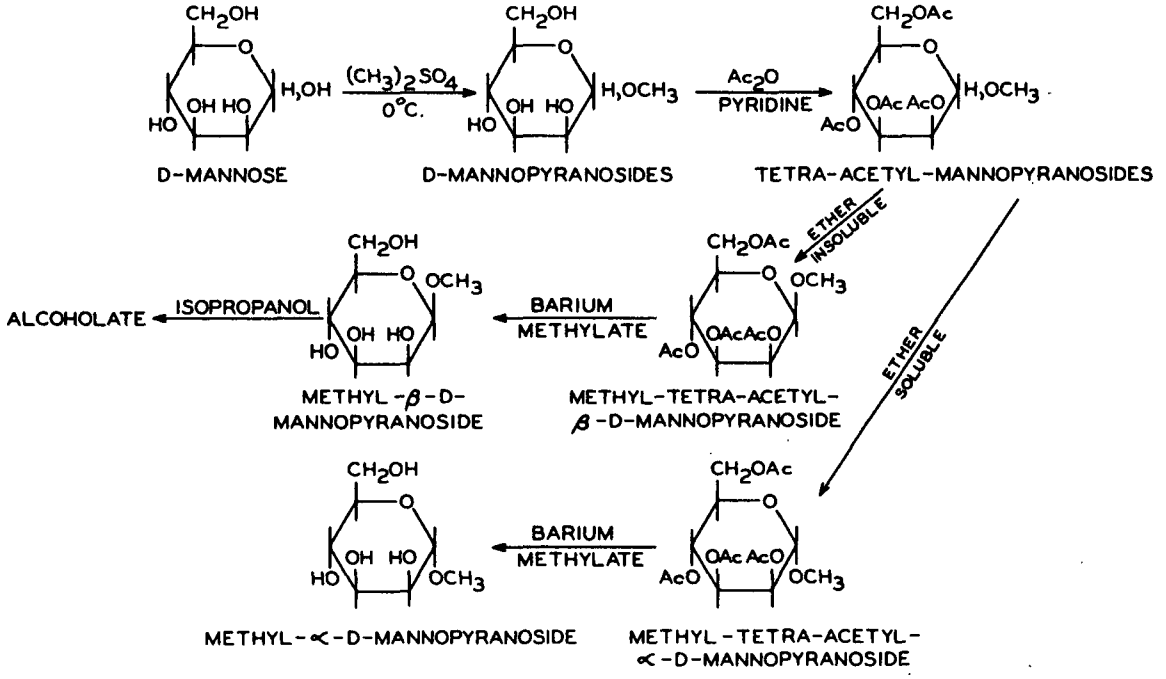


Figure 3. Production of the Glycosides of D-mannose by Direct Alkylation

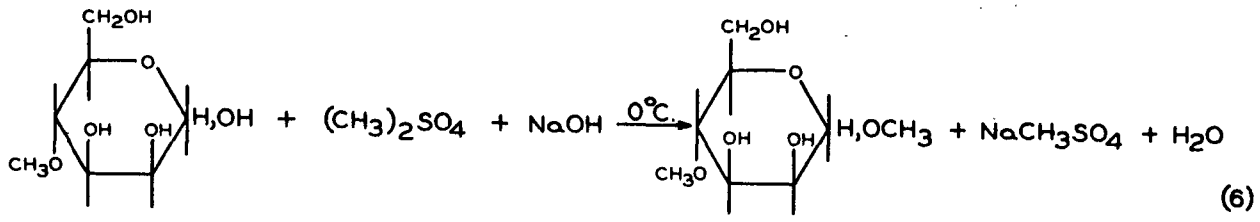
TABLE I

MELTING POINTS OF PRODUCTS FROM THE MANNOSIDE PREPARATION

Compound	Melting Point, °C. Experimental	Literature (52)
Methyl-tetra acetyl-β-D-mannoside	158-160	159-160
Methyl-β-D-mannoside isopropyl alcoholate	70-72	74-75
Methyl-β-D-mannoside	glass	glass
Methyl α-D-mannoside	186-190	193-194

DIRECT ALKYLATION TO GIVE THE GLYCOSIDES OF 4-O-METHYL-D-MANNOSE

The reaction with 4-O-methyl-D-mannose is given in Equation (6):



(6)

4-O-METHYL-D-MANNOSE

4-O-METHYL-D-MANNOSE GLYCOSIDES

The experimental details are given in the following sections.

Initial Formation Procedures

For the initial run, five grams (0.026 mole) of the 4-O-methyl sugar were dissolved in 26 ml. of water (1 mole/liter), and dimethyl sulfate (0.027 mole) was added dropwise to the sugar solution, with 9.7N sodium hydroxide (0.262 mole) added to keep the solution alkaline. The temperature of the reaction varied from 2-4°C., while the pH of the solution was in the 10.5-11.5 range.

Chromatographic analysis of the glycoside mixture showed a large portion of the 4-O-methyl-D-mannose was unreacted. From the relative size of the spots on the chromatogram, it was estimated about half of the 4-O-methyl-D-mannose reacted. Of this half, approximately one quarter was the β-glycoside. The analysis showed only traces of unwanted, higher methylated compounds.

The glycoside mixture was then processed to give the acetates of the various compounds in the mixture. However, crystallization attempts proved unsuccessful, and the acetate groups were removed by the catalytic barium methylate method.

Before attempting further work with the glycoside mixture, the relative amount of unreacted sugar in the mixture was decreased by repetition of the dimethyl sulfate procedure. Conditions and concentrations were the same as in the initial run. After removal of the salts by pyridine, the mixture was not acetylated, but, after removing the pyridine, was dissolved in 2-butanone. Chromatographic analysis of the 2-butanone solution showed that most of the 4-O-methyl-D-mannose had reacted. As expected, increased amounts of higher methylated materials were also found.

A small portion of the mixture was hydrolyzed, analyzed chromatographically, and found to contain dimethyl mannose, with traces of the trimethyl compound. However, an exceedingly heavy amount of 4-O-methyl-D-mannose was also present. Therefore, although the amount of higher methylation was greater than might have been desired, only a relatively small portion of the total 4-O-methyl-D-mannose was involved.

A series of different solvents was used in an attempt to obtain a crystalline product from the glycosidic mixture. Isopropanol, 2-butanone, ethanol, and dioxane were among the solvents tested. Mixed solvents were also tried, that is, the addition of a poor solvent to a solution of the glycoside mixture in a good solvent. Warm as well as cold temperatures were also used. No crystalline product was obtained, and it was necessary to use column chromatography to obtain a pure form of the β-glycoside.

The preparation of the column and the application procedure are described in Appendix I. Sample collection was started after 450 ml. of developer had passed through the column. The average flow rate during the run was 160 ml./hr. Individual fractions of 20 to 25 ml. each were collected and analyzed for carbohydrate.

The orcinol test (53) was used to locate the carbohydrate material. The method used involved reaction at 100°C. for twenty minutes with a 0.2% solution of orcinol in 75% sulfuric acid, which produced a yellow color if a carbohydrate was present. The optical density of the solution was determined at 525 mμ, using a Beckman Spectronic 20 colorimeter. Once the carbohydrate was located, paper chromatography was used to analyze the appropriate fractions.

The initial separation resulted in overlapping of some compounds, and the fractions containing the 4-O-methyl-β-mannoside were combined, concentrated, and rerun through the column. This second run was made in the same manner as the first except for the sample size, which was cut to 20 ml., and the flow rate, which was lowered to 90 ml./hr. Figure 4 shows the results of the analytical tests. The 4-O-methyl-β-mannoside was found pure in tubes 29-31, and these fractions were combined and concentrated to a sirup. The respective fractions containing the 4-O-methyl-α-mannoside and the unreacted 4-O-methyl-D-mannose were also combined and concentrated.

Production Runs Yielding the 4-O-methyl-β-mannoside

Two additional runs were made to prepare the methyl glycosides of 4-O-methyl-D-mannose. In each run, 4.0 g. (0.0206 mole) of 4-O-methyl-D-mannose were processed by the dimethyl sulfate—sodium hydroxide method. The procedure was the same as the initial run, except for the use of ethanol in place of pyridine in the final salt-removal step.

The reaction mixtures were analyzed by paper chromatography. Both the α- and β-glycosides were found, together with some unreacted 4-O-methyl-D-mannose and small amounts of di- and trimethylated mannose. During the second run, the pH of the reaction mixture was accidentally increased from the desired 10.5-11.0

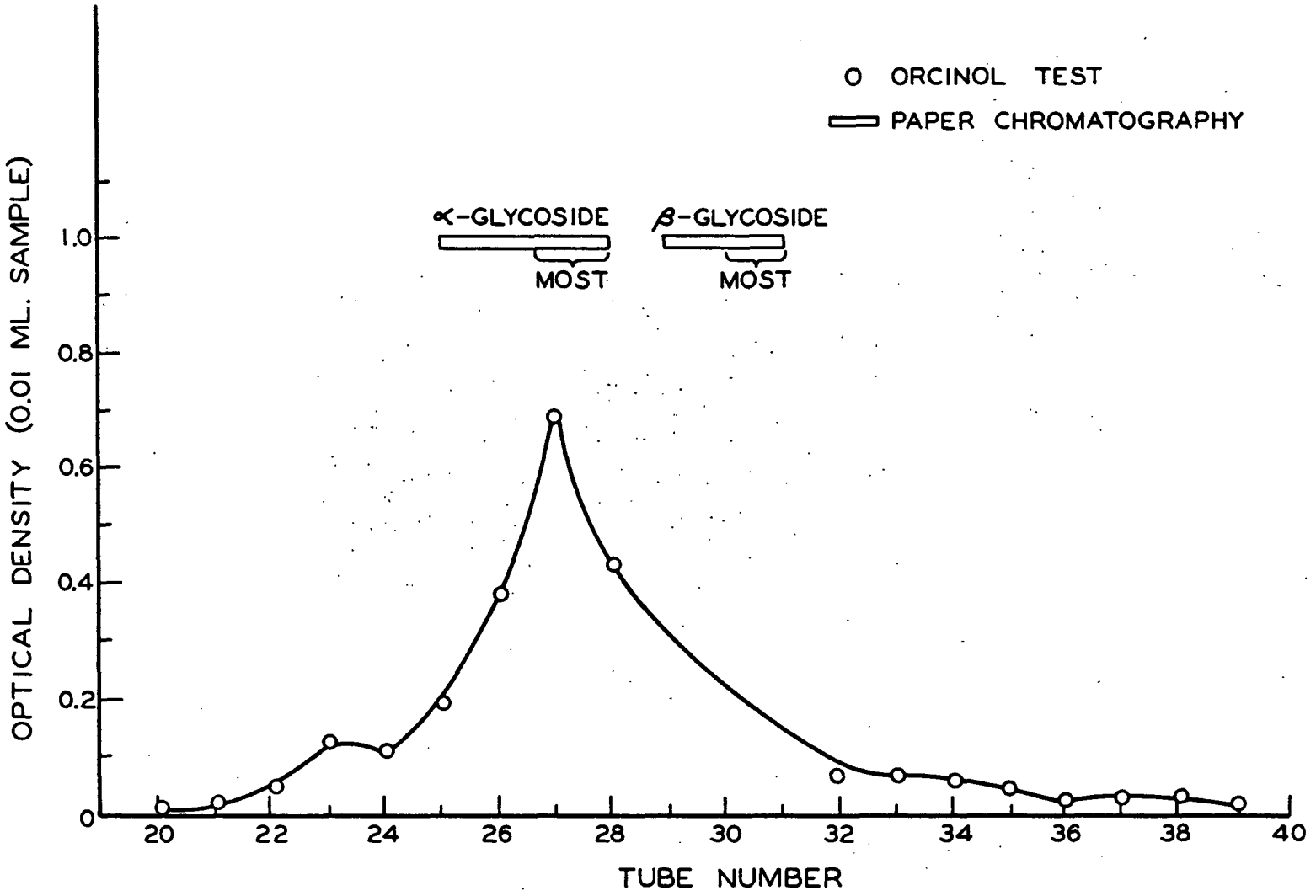


Figure 4. Results of Column Chromatographic Separation

pH range to 12.5. As expected, this caused an increase in the amount of direct methylation, with a corresponding decrease in glycoside production.

Before the second production run could be made, it was necessary to recover 4-O-methyl-D-mannose from previous runs. Because a major portion of the 4-O-methyl-D-mannose from previous runs was present as glycosides, acid hydrolysis (2N H_2SO_4 at 100°C. for one hour) was used to give the free sugar. Besides 4-O-methyl-D-mannose, paper chromatographic analysis of the hydrolysis product showed the presence of considerable amounts of dimethyl mannose, together with traces of mannose and higher methylated products. The hydrolyzed mixture was combined with the remaining mother liquid from the initial 4-O-methyl-D-mannose production and passed through the cellulose column. Good separation was obtained, yielding pure 4-O-methyl-D-mannose which readily crystallized from ethanol and was used in the second production run.

The glycosides were separated by cellulose column chromatography, and the various fractions were located by use of the orcinol test and identified by use of paper chromatography. In both runs, reasonable separation was obtained on one pass through the column. The 4-O-methyl-β-D-mannoside obtained from the two runs was combined and rerun through the column to give the chromatographically pure product. The α-glycoside readily crystallized from 2-butanone, and as can be seen in Table II, has physical properties that checked closely with published values.

TABLE II

YIELDS AND PHYSICAL CONSTANTS OF THE GLYCOSIDES OF 4-O-METHYL-D-MANNOSE

Compound	Total Yield, g.	Specific Rotation, °		Melting Point, °C.	
		$[\alpha]_{589}^{20}$ Exptl.	$[\alpha]_{589}^{20}$ (49)	Exptl.	(49)
Methyl-4-O-methyl-α-D-mannopyranoside	1.9	84.3	83.9	100-101	101-102
Methyl-4-O-methyl-β-D-mannopyranoside	0.8	-58.3	--	--	--

Determination of the Yield of Methyl-4-O-methyl-β-D-mannopyranoside

As attempts to crystallize the 4-O-methyl-β-mannoside failed, spectrophotometric measurements were used to determine the amount of material available. Because the α-glycoside of 4-O-methyl-D-mannose was used as the reference substance, a colorimetric test was needed where the conditions were such that hydrolysis of the glycosides occurred, and the color-producing reaction was with the free sugar.

The orcinol test with its highly acidic solution and high temperature met these requirements and was used in the concentration determinations. The assumption that the reaction occurs with the free sugar was checked by comparing known solutions of 4-O-methyl-D-mannose and the 4-O-methyl-α-mannoside. Agreement within 1% was obtained.

In all colorimetric measurements, the Beckman DU spectrophotometer was used to measure optical densities. The wavelength of maximum absorption was found to occur at 424 mμ and was used in all measurements.

The reference curve of optical density versus concentration was determined with the 4-O-methyl-α-mannoside as the reference compound. The volumetric measurements were made using a semimicro buret with an estimated accuracy of ± 0.002 ml. The orcinol test was run as previously described. A 0.1-ml. aliquot of the 25 ml. of the β-glycoside solution from the two production runs was diluted 1:100 and processed in the same manner. Duplicate tests were run at each point, with the results given in Fig. 5.

From the values given in Fig. 5, the yield of the 4-O-methyl-β-mannoside from the two production runs was calculated to be 0.58 g. A similar procedure

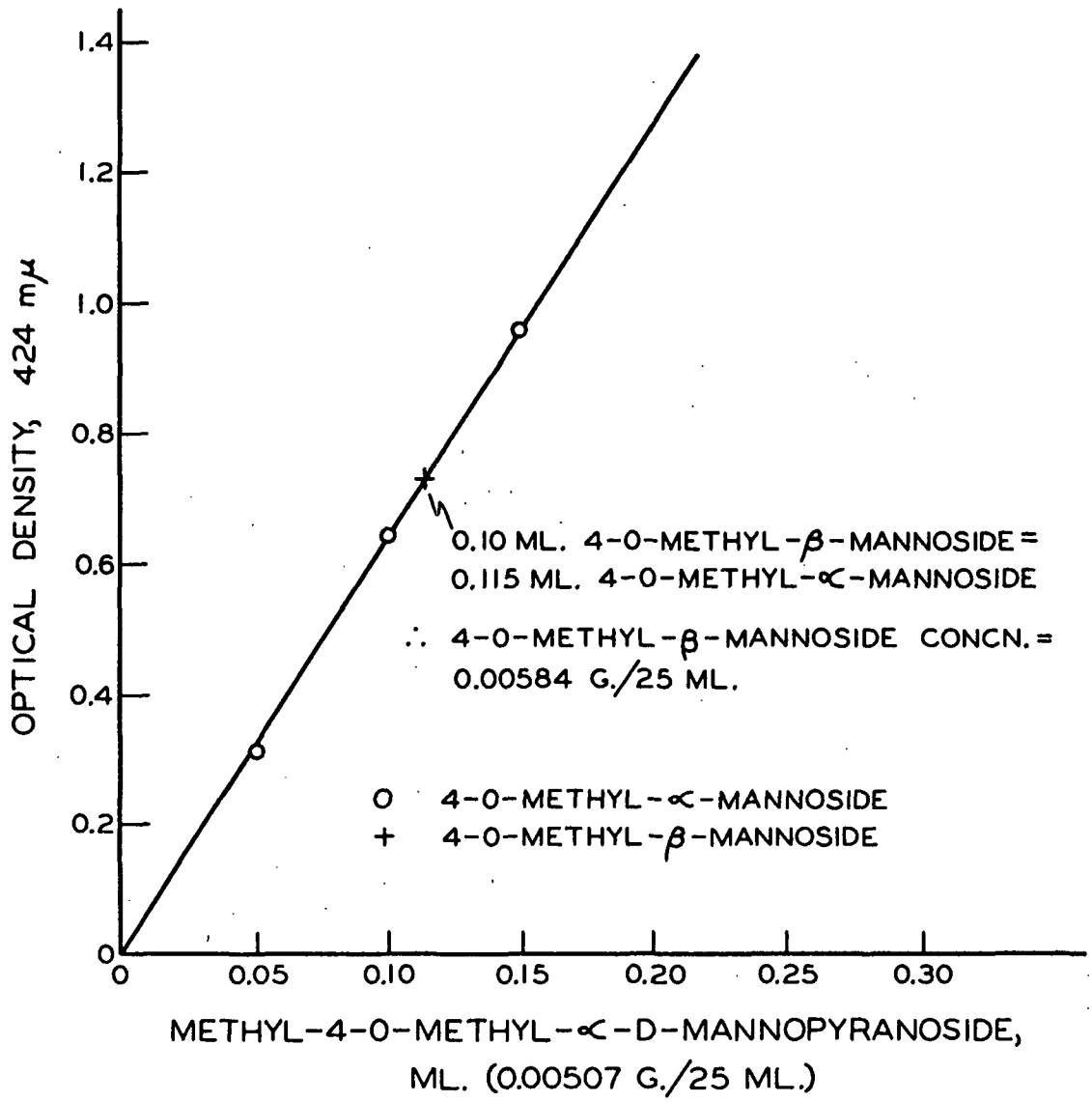


Figure 5. Determination of Methyl-4-O-methyl-β-D-mannopyranoside Concentration Using Methyl-4-O-methyl-α-D-mannopyranoside as the Reference Compound

was used to determine that 0.25 g. of the β-glycoside was obtained in the initial formation study. Hence, a combined total of 0.83 g. of the 4-O-methyl-β-mannoside was obtained for use in the study of the borate complex. While this amount was lower than hoped for, it was sufficient for the study.

Characterization of Methyl-4-O-methyl-β-D-mannopyranoside

Isolation of the β-glycoside has not been previously reported. The purity of the compound as well as its tentative identification as the β-glycoside was determined by paper chromatography. The compound was nonreducing, and on acid hydrolysis gave only 4-O-methyl-D-mannose. A negative specific optical rotation comparable to that estimated by use of Hudson's isorotation rules (51) was found. The estimated value for the specific optical rotation was -55° (see Appendix I), while the actual experimental value was -58.3°. The compound thus proved to be the β-glycoside of 4-O-methyl-D-mannose.

BORATE COMPLEX STUDY

MATHEMATICAL BASIS

GENERAL DEFINITIONS

A complex is defined as a species formed by the association of two or more simpler compounds, each capable of independent existence (54). The stability of a complex can be expressed quantitatively in terms of its stability constants. If the activity coefficient of each species is held constant, the stoichiometric stability constants are defined by

$$\beta_n = [BA_n]/[B][A]^n \qquad (7),$$

where β_n is the over-all stability constant for the reaction

$$B + nA \rightleftharpoons BA_n \qquad (8),$$

and the brackets indicate equilibrium concentrations; and

$$K_n = [BA_n]/[BA_{n-1}][A] \qquad (9),$$

where K_n is the step stability constant for the reaction

$$BA_{n-1} + A \rightleftharpoons BA_n \qquad (10).$$

The over-all and step stability constants are related by

$$\beta_n = K_1 \cdot K_2 \cdot \cdots \cdot K_n = \prod_{\pi}^{n} K_n \qquad (11).$$

If more than one complex forms, it is convenient to employ the following relation, concerning the total amount complexed.

$$K_T = \frac{[BA][BA_2]\cdots\cdots[BA_n]}{[B]^n\,[A]^{\sum_1^n n}} \qquad (12).$$

where K_T is the total stability constant for the reaction

$$nB + \sum_1^n n\,A \rightleftharpoons BA + BA_2 + \cdots\cdots + BA_n \qquad (13).$$

The total stability constant is related to the over-all stability constants by

$$K_T = \prod^n \beta_n \qquad (14).$$

The above definitions are all based on the formation of a mononuclear complex, with B the central group and A the ligand.

MEANS OF OBTAINING STABILITY CONSTANTS

Rossotti and Rossotti (54) have reviewed both the theory and techniques of the determination of stability constants. The majority of the methods revolve around a determination of the average number of ligands bound to one central atom as a function of the free concentration of the ligand. The free concentration can sometimes be determined by direct physical measurements, such as the potentiometric determination of the hydrogen ion concentration, while in other cases competitive reactions can be used. Once the desired free concentration is known, a variety of graphical methods is available for determining the stability constants (54, 55).

If the free concentration of a reacting species cannot be determined, other methods, involving the use of some physical property of a solution containing the complex, are available. A number of properties Y of a solution can be defined by

$$Y = k\left(\sum_{o}^{n} x_n [BA_n] + x_a f\right) \qquad (15),$$

where x_n and x_a are the intensive factors corresponding to the species BA_n and A, f is the free concentration of A at equilibrium, and k is an instrumental constant. The above relation is the basis for a number of methods widely used to determine the formulae and stability constants of complexes. While these methods go by a variety of names, all are similar in nature to the method of continuous variations as initially applied by Job (56).

Method of Continuous Variations

In essence, the continuous variation methods employ the measurement of some property Y of a solution as a function of the molar ratio of the reacting species. A maximum or minimum occurs at the molar ratio corresponding to the composition of the complex. This change becomes more evident if ΔY, that is, the difference between the experimental value of Y and the value calculated assuming no complex formation, is plotted as a function of the initial molar ratio of the reacting species. The mathematics for the above method have been developed in detail by several workers (54, 56-58), and will not be repeated here.

One important fact must be noted. The method of continuous variations was developed for and is exact for the determination of only one complex. Several attempts have been made to extend the method for a quantitative study of more than one complex, but none have met with much success (54). However, several qualitative extensions have been made. Giles and co-workers (58-62) made use of the continuous variations method to study apparent hydrogen bonding in a wide variety of compounds. Nelson (63) used similar methods to obtain qualitative indications of many metal complexes and has extensively reviewed the application

of continuous variation measurements to inorganic systems. Both Giles and Nelson employed refractive index measurements in their studies on complex formation. Reeves and Bragg (64) have made use of the continuous variations method in a study of the cuprammonium-diol complex, using optical absorbance as a physical property. It must be stressed that the presence of more than one complex invalidates most quantitative uses of the method, but if due caution is used, the method is of qualitative value.

Simultaneous Equations Method

The simultaneous equations method of determining stability constants has not received much use, primarily due to the difficulty of handling a large number of equations (54). However, if the number of complexes is small, Equation (15) can be solved directly for the concentrations of the various species present at equilibrium. To use Equation (15), the several intensive factors must be known, and n sets of independent equations must be experimentally determined, using different physical properties for Y.

Jones and Innes (57) have discussed the various physical properties that can be used as defined in Equation (15). In general, these properties were divided into those characteristic of the ions or molecules themselves, and those that, while characteristic of the solution, can be apportioned to the various species in solution on a basis of their concentration. In the first category such properties as light absorption, optical rotation, and NMR were of use. In the latter category such factors as magnetic susceptibility and refraction were of value. All of these factors gave linear relations in dilute solutions and are suitable properties for use in Equation (15).

APPLICATION TO THE BORATE SYSTEM

The borate-carbohydrate system presented several obstacles to determining complex concentrations or stability constants, the biggest factor being the similarity between the unreacted species and the complex that forms. For example, if a competitive reaction was used to determine the concentration of the free carbohydrate, there is a great likelihood that the complex will also react because of the similar structures of the carbohydrate and the complex. Attempts to determine the free borate concentration run into similar troubles. Therefore, the simultaneous equations method was used to determine the concentration of all the components in the carbohydrate-borate system.

Because two types of complex formed, it was necessary to obtain two equations with the concentration of the complexes as unknowns. Once the concentrations were known, the stability constants were calculated directly. The continuous variations method was incorporated into the procedure to give a qualitative indication of the types of complex present. Before entering into a discussion of the mathematical basis for these procedures, the experimental conditions which have a bearing on the mathematics must be discussed.

Experimental Conditions Related to the Mathematical Development

As has been mentioned, the pH of the experimental solutions must be kept above 11.0 both to avoid the interference of polyborate anions and to ensure complete ionization of the boric acid. Because it was also necessary to maintain constant ionic strength, the initial concentration of potassium hydroxide was set at a constant value. This concentration was such that a slight excess of potassium hydroxide was present at the highest borate concentration. Consequently, as the borate concentration decreased, the excess of alkali increased. For reasons

which will be discussed later, the initial potassium hydroxide concentration was maintained at 0.105M.

The initial concentration of alkali fixed the ionic strength of the solution. The addition of a carbohydrate results in a 1:1 production of the monovalent complex anion from the monovalent borate anion and does not change the ionic strength. The addition of boric acid results in a 1:1 production of monovalent borate anion from the monovalent hydroxide anion, with a slight increase in the hydronium ion concentration. This increase in the hydronium ion concentration was of the order of 10^{-11} mole/liter and was neglected. The cation concentration was then effectively equal to the potassium ion concentration and remained constant throughout the experiment. Since only monovalent anions were present and their amount remained constant, the ionic strength also was constant.

Development of the General Equation for ΔY

If a physical property, Y, of a solution is a linear function of the concentration of a compound, A, the relation is given by

$$Y = a[A] + Y_o \qquad (16),$$

where a is a constant, [A] is the molar concentration of A, and Y_o is the Y value of the pure solvent. If more than one compound is present in a dilute solution, an additive relation can be assumed, and the value of Y is given by

$$Y = a[A] + b[B] \cdots\cdots z[Z] + Y_o \qquad (17),$$

where a, b, $\cdots$z are the appropriate constants corresponding to the compounds A, B, $\cdots\cdots$ Z.

On applying the relation to the alkaline borate system, $\underline{Y}$ is given by

$$Y = a[D] + b[KB] + c[KBD] + d[KBD_2] + e[KOH] + Y_o \qquad (18).$$

Here,

D = a carbohydrate containing a cis-hydroxyl pair

K = the potassium cation, K^+

B = the borate anion, $B(OH)_4^-$.

Equation (18) is the general equation defining the variation of a general physical property of a solution as a function of the concentration of the various components of the borate-carbohydrate system.

Because the potassium ion takes no part in the reaction, for the sake of brevity, it will be omitted in the following sections. It will always be assumed that the necessary potassium ions are present to preserve the electrical neutrality of the solution.

It now remains to simplify the general equation to a more workable form. From a general mass balance,

$$[D] = [D_o] - [BD] - [BD_2] \qquad (19),$$

$$[B] = [B_o] - [BD] - [BD_2] \qquad (20),$$

and

$$[KOH] = [KOH_o] - [B_o] \qquad (21).$$

The subscript o refers to the initial concentration of material added to the solution. Since the initial concentration of potassium hydroxide was held constant, $[KOH_o]$ represents a constant value. Also, the use of excess alkali enables $[B_o]$ to be defined as the initial amount of boric acid added to a solution.

On substitution of the values given in Equations (19)-(21) into Equation (18), the following equation, expressing $\underline{Y}$ as a function of the complex concentrations and the initial experimental conditions, is obtained.

$$Y = (c - a - b)[BD] + (d - 2a - b)[BD_2] + a[D_o] + (b - e)[B_o] + e[KOH_o] + Y_o \quad (22).$$

One additional experimental condition must be incorporated into Equation (22). In the continuous variations method used in this study, the sum of the molar concentrations of $\underline{B_o}$ and $\underline{D_o}$ is set at some fixed value, $\underline{M}$. That is,

$$[D_o] + [B_o] = M \quad (23).$$

On substitution of Equation (23) into Equation (22), the final relation is obtained,

$$Y = (c - a - b)[BD] + (d - 2a - b)[BD_2] + (a + e - b)[D_o] + (b - e)M + e[KOH_o] + Y_o \quad (24).$$

Equation (24) defines the variation of $\underline{Y}$ during use of the continuous variations method of studying the borate-carbohydrate complex. It should be kept in mind that defining $[\underline{D_o}]$ also defines $[\underline{B_o}]$ through the relation given in Equation (23).

If no complex formation occurs, Equation (24) becomes

$$Y_{nc} = (a + e - b)[D_o] + (b - e)M + e[KOH_o] + Y_o \quad (25).$$

It will be convenient to express the experimental results in terms of $\Delta\underline{Y}$, that is, the change in $\underline{Y}$ due to complex formation. By definition,

$$\Delta Y = Y_{nc} - Y \quad (26).$$

Therefore, by use of Equations (24) and (25),

$$\Delta Y = (c - a - b)[BD] + (d - 2a - b)[BD_2] \quad (27).$$

Equation (27) contains two unknown quantities, [BD] and $[BD_2]$, and one experimental value, ΔY. This equation will serve as the working equation in this study.

Determination of Constants

Before the general working equation can be used, the various constants must be determined. The value of a and e can be determined separately from the individual compounds. The appropriate relations are given by

$$Y = e[KOH] + Y_o \quad (28),$$

and

$$Y = a[D_o] + e[KOH_o] + Y_o \quad (29).$$

Because an excess of alkali was used at all times, the determination of a was made in excess alkali. It will be remembered that $[KOH_o]$ is a constant term.

For the determination of b, the following relation holds.

$$Y = b[B] + e[KOH] + Y_o \quad (30).$$

On substitution of Equation (21) and the fact that $[B] = [B_o]$, one obtains

$$Y = (b - e)[B_o] + e[KOH_o] + Y_o \quad (31).$$

Therefore, b can be calculated from the slope of a Y versus $[B_o]$ plot.

The values of c and d cannot be so directly determined. However, by suitable adjustment of experimental conditions, these values can be calculated.

Determination of c

If conditions are set so that [B] >> [D], it can be assumed that only the BD complex forms and that all of D is complexed. In this case,

$$[BD] = [D_o] \quad (32),$$

$$[D] = [BD_2] = 0 \quad (33),$$

and

$$[B] = [B_o] - [BD] = [B_o] - [D_o] \quad (34).$$

The values given in Equations (21) and (32)-(34) can be substituted in the general Equation (18). On rearrangement of terms, one obtains

$$Y = (c - b)[D_o] + (b - e)[B_o] + e[KOH_o] + Y_o \quad (35).$$

When $[\underline{B}_o]$ is held constant, Equation (35) becomes linear with a slope of ($\underline{c}$ - $\underline{b}$). Therefore, $\underline{c}$ can be determined from a plot of $\underline{Y}$ versus $[\underline{D}_o]$.

Determination of $\underline{d}$

If conditions are set so that $[\underline{D}] >> [\underline{B}]$, it can be assumed that only the BD_2 complex forms and that the concentration of B is zero. In this case,

$$[BD_2] = [B_o] \quad (36),$$

$$[B] = [BD] = 0 \quad (37),$$

and

$$[D] = [D_o] - 2[BD_2] = [D_o] - 2[B_o] \quad (38).$$

On substitution of the values given in Equations (21) and (36)-(38) into Equation (18), followed by rearrangement of terms,

$$Y = (d - 2a - e)[B_o] + a[D_o] + e[KOH_o] + Y_o \quad (39).$$

If $[\underline{D}_o]$ is held constant, Equation (39) is linear with a slope of ($\underline{d}$ - 2$\underline{a}$ - $\underline{e}$). Since $\underline{a}$ and $\underline{e}$ are known, a plot of $\underline{Y}$ versus $[\underline{B}_o]$ will yield $\underline{d}$ from the slope of the curve.

In all of the determinations, the least squares method was used to determine the slopes, which were checked by use of the statistical F-test.

Therefore, the various constants can be experimentally determined, and Equation (27) can be used to give the necessary number of equations needed to calculate the concentrations of the BD and BD_2 complexes.

Verification of the Coefficients in the ΔY Equation

Ratio Test

As will be seen, the concentrations of BD or BD_2 became negligible near the ends of the concentration range used in the continuous variations procedure. That is, in most cases the concentration of BD_2 was zero at low $[D_o]$ values, while the [BD] value was usually close to zero at the high $[D_o]$ values. Because of this, it was possible to check the coefficients used in the ΔY working equations with the ratio test, making use of the continuous variations data at either end of the concentration scale. This ratio check was of special value in cases where the accuracy of a constant was open to some doubt due to a limited amount of available data.

Let the working equations for calculation of [BD] and $[BD_2]$ be represented in the general case as,

$$\Delta Y_1 = F[BD] + G[BD_2] \qquad (40),$$

and

$$\Delta Y_2 = H[BD] + I[BD_2] \qquad (41)$$

where F, G, H, and I represent the coefficients calculated from the various constants. If the concentration of BD is negligible,

$$\Delta Y_1/\Delta Y_2 = G/I \qquad (42).$$

This ratio can then be compared with the ratio of actual values of $\Delta\underline{Y}_1$ and $\Delta\underline{Y}_2$ at high $[\underline{D}_o]$ values. This provides a check on the $[BD_2]$ coefficients of the working equations.

Similarly, if $[BD_2]$ is near zero,

$$\Delta Y_1/\Delta Y_2 = F/H \qquad (43).$$

This can be compared with the ratio of the actual values of $\Delta\underline{Y}_1$ and $\Delta\underline{Y}_2$ at low $[\underline{D}_o]$ values, providing a check on the [BD] coefficients.

While the ratio test was initially intended only as a rough check, in practice it gave unexpectedly close comparisons. Hence, it serves as a valuable aid in judging the reliability of the coefficients.

Back-Calculation of $[\underline{D}_o]$

In certain cases the coefficients for BD or BD_2 in the $\Delta\underline{Y}$ working equation was found to be small. Under these circumstances, Equation (24) can be used to treat $[\underline{D}_o]$ as an unknown. If one of the terms related to a complex drops out, Equation (24) contains $[\underline{D}_o]$ and the concentration of one of the complexes as unknowns. Hence, with two independent physical properties, $[\underline{D}_o]$ can be calculated directly and compared with the actual experimental value of $[\underline{D}_o]$. In cases where this was possible, this procedure served as the best means of checking both the working equations and the accuracy of the concentration determinations.

Physical Properties Used to Follow Complex Formation

At least two different properties are needed, with a third property desirable for checking the results. Of the various methods available, refractive index and

optical rotation were selected for use. Both can be determined quickly and conveniently. Both have the added advantage of being a function of the wavelength of the light used in making the measurement. This is of value because additional equations can be obtained by changing the wavelength of light at which the measurements are taken.

Refractive Index and Optical Rotation Working Equations

When refractive index is used as a physical property of a solution, Equation (27) becomes

$$\Delta R = (c - a - b)[BD] + (d - 2a - b)[BD_2] \qquad (44),$$

where R is the refractometer reading.

When optical rotation is used as a physical property, several constants have values of zero, and Equation (27) becomes

$$\Delta\alpha = (c - a)[BD] + (d - 2a)[BD_2] \qquad (45),$$

where α is the observed optical rotation.

It should be made clear that the constants in Equations (44) and (45) are not the same in numerical value, but depend on the physical property with which they are associated. However, the constants do refer to the same component, no matter what physical property is used. For example, a refers to the change in the measured physical property due to a change in the free carbohydrate. However, its numerical value will depend on the physical property being measured.

Equations (44) and (45) can be solved simultaneously to calculate the concentrations of BD and BD_2 in a solution. If the optical rotation values are

determined at two different wavelengths, an additional equation can be obtained to serve as a check. In effect, three equations in two unknowns can be obtained.

Treatment of the ΔR and $\Delta\alpha$ Values

The experimental values of R and α are obtained as a function of the ratio of the initial concentration of carbohydrate to borate. They are converted to ΔR and $\Delta\alpha$ values through use of Equations (25) and (26). Before the values of ΔR and $\Delta\alpha$ are used to calculate the BD and BD_2 concentrations, polynomials of from third to fifth power are fitted to the data by use of a multiple linear regression to average the scatter and minimize the deviations due to experimental error. It is not intended that any theoretical significance be attached to the polynomial.

Generally, all of the polynomials gave a good fit in regions where the data points were concentrated. In most cases this region was from a $[D_o]$ value of 0.03 to 0.07 mole/liter. As lower power polynomials gave the best averaging effect, the third-power polynomial was used in most cases. In a few situations a higher power polynomial was used to give a more likely fit at the ends of the curve. Some liberties could be taken in choosing the best fitting polynomial as the calculations of stability constants were made from the middle of the curve, where all polynomials gave similar results.

The plot of ΔR or $\Delta\alpha$ versus the initial molar concentration ratio is also used to indicate the molar ratio of D to B in the complex from the maximum points in the curve. The working equations for ΔR and $\Delta\alpha$ are needed in this step as they indicate the relative magnitude of the contributions of each complex to the ΔR and $\Delta\alpha$ values.

Calculation of Stability Constants

Once the concentrations of BD and BD_2 are known, the free equilibrium concentration of D and B can be calculated from Equations (19) and (20). With the concentration of all of the components of the system known, the stability constants can be directly calculated.

For the purposes of this study several equilibria and their corresponding stability constants must be considered. These are defined by:

$$B + D \overset{\beta_1}{\rightleftharpoons} BD \tag{46},$$

$$\beta_1 = [BD]/[B][D] \tag{47},$$

$$BD + D \overset{K_2}{\rightleftharpoons} BD_2 \tag{48},$$

$$K_2 = [BD_2]/[BD][D] \tag{49},$$

$$B + 2D \overset{\beta_2}{\rightleftharpoons} BD_2 \tag{50},$$

$$\beta_2 = [BD_2]/[B][D]^2 \tag{51},$$

$$2B + 3D \overset{K_T}{\rightleftharpoons} BD + BD_2 \tag{52},$$

$$K_T = [BD][BD_2]/[B]^2[D]^3 \tag{53}.$$

The following relations between the stability constants also hold:

$$K_2 = \beta_2/\beta_1 \tag{54}$$

$$K_T = \beta_2 \cdot \beta_1 \tag{55}.$$

The values of β_1 and β_2 are the over-all stability constants for the formation of the BD and BD_2 complexes, respectively, and serve to compare the relative complexing

abilities of the model compounds. It should be noted that β_2 represents the formation of BD_2 from D and B, and not from BD and D. Hence, β_2 can be greater than β_1 without indicating that the BD_2 complex is the dominant species. K_2 is the step stability constant and will be of use in comparing the relative dominance of BD or BD_2. Under the conditions of this study, a K_2 value of approximately 25 indicates equal amounts of BD and BD_2. Higher values show BD_2 dominance, while lower values indicate BD dominance. K_T is the total stability constant and serves to compare the total complexing ability of the model compounds.

INTRODUCTORY INVESTIGATIONS

Before the refractive index and optical rotation experiments were run, the pH method was used for a qualitative indication of complex formation. Several potentiometric titrations of boric acid are shown in Fig. 6 and 7, with and without the presence of a polyhydric material. Mannitol, mannose, galactose, and the α-galactoside were used to demonstrate complex formation, with the α-galactoside representing the model compounds. The other model compounds were not used due to the limited amount of each available. As expected, the addition of a polyhydroxyl compound caused an increase in the hydronium ion concentration, with a resulting decrease in the pH of the solution. No change in the end point of the titration was found, and the assumption that boric acid is completely ionized above a pH value of 11.5 is still valid in the presence of a polyhydroxyl compound. These results not only served as initial indicators of complex formation, but also demonstrated that the type of carbohydrate affects the degree of complex formation.

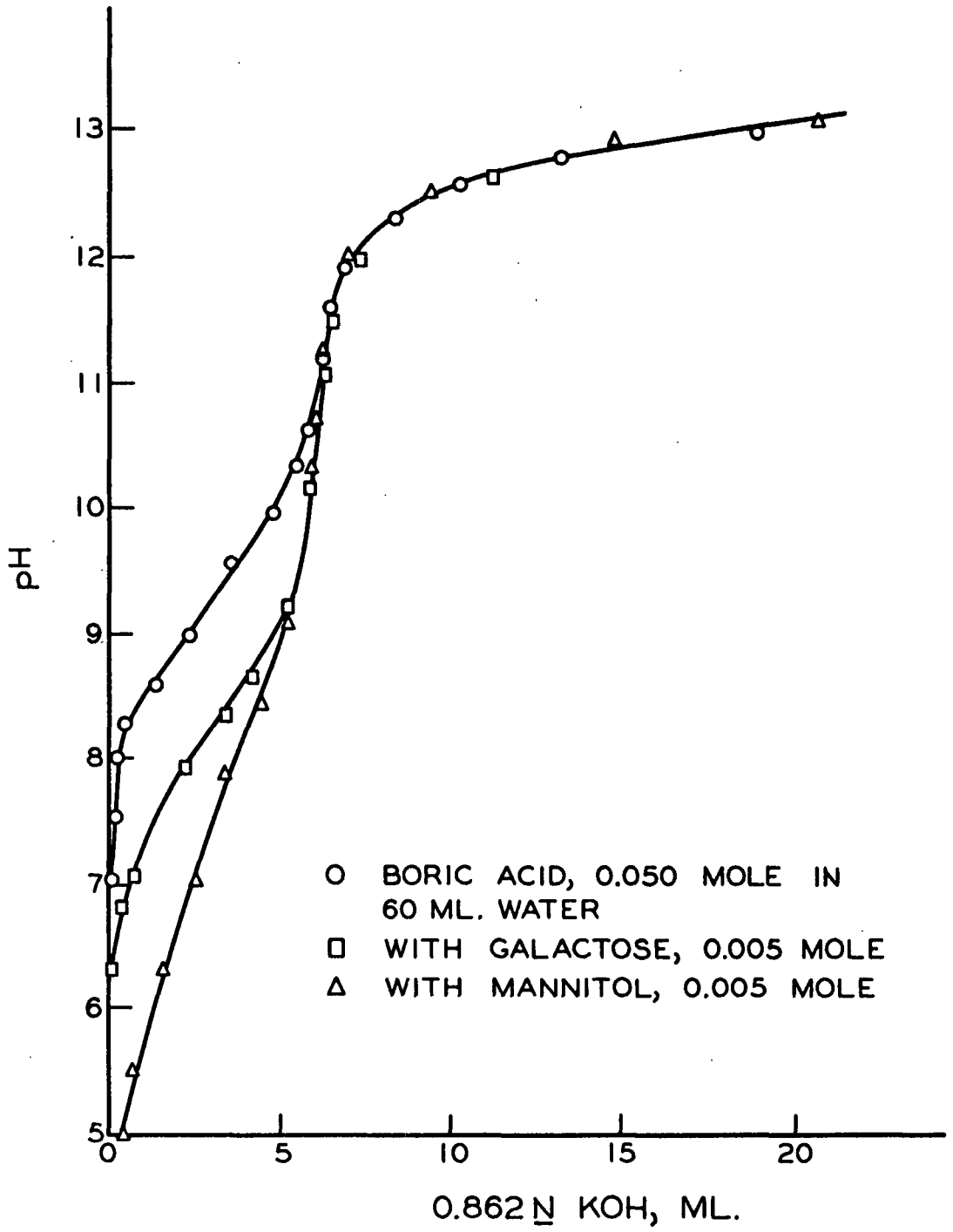


Figure 6. The Effect of Polyhydric Compounds on the Titration Curve of Boric Acid

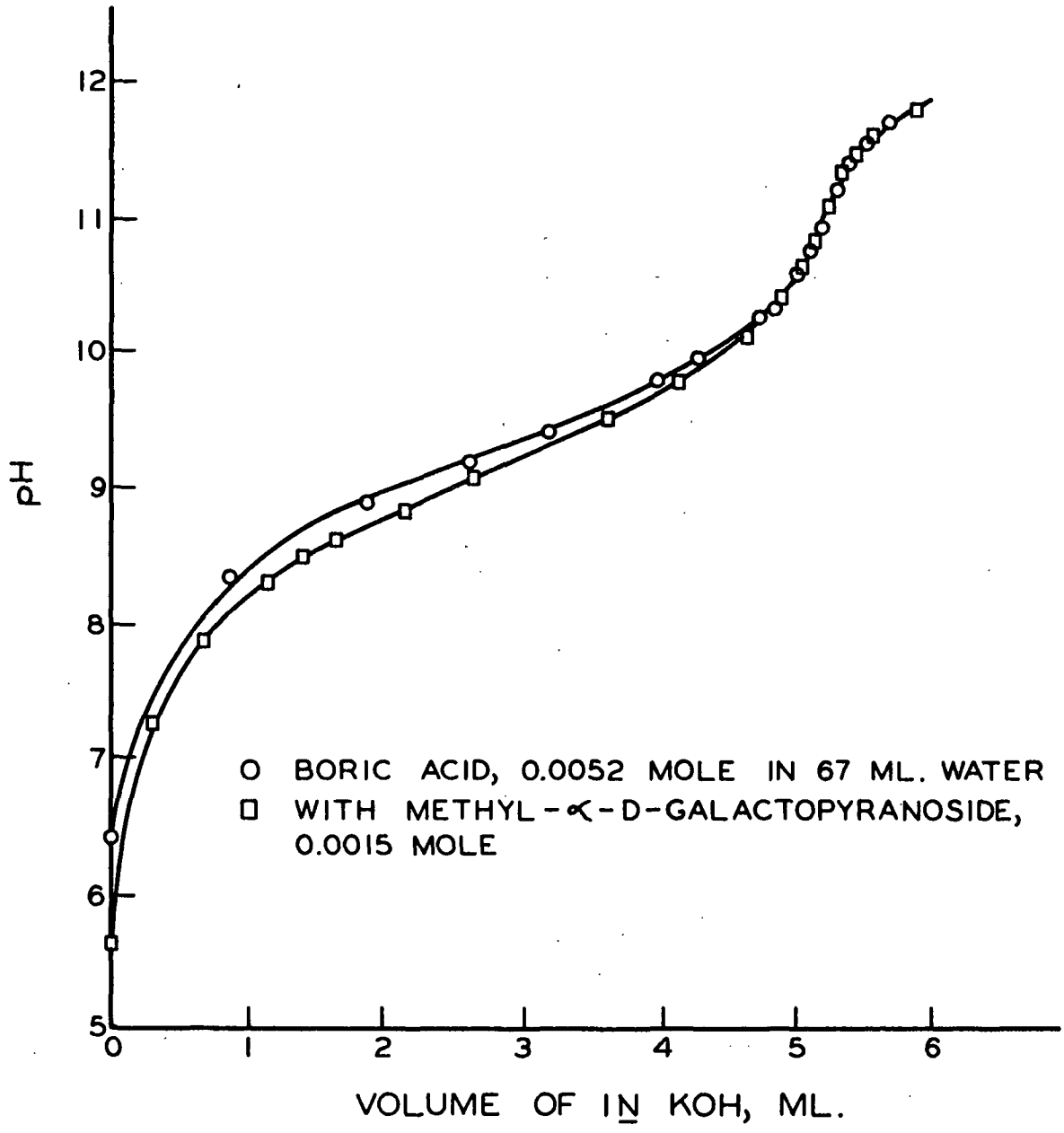


Figure 7. Effect of Methyl-α-D-galactopyranoside on Titration of Boric Acid

EXPERIMENTAL PROCEDURE

GENERAL CONDITIONS OF MAIN STUDY

It was found that 10 ml. of solution were necessary to carry out the physical measurements. From a consideration of the amount of each model compound available, the maximum concentration of carbohydrate was set at 0.100M. Because the continuous variations method sets the total amount of borate plus carbohydrate at a constant value, the maximum borate concentration must also be 0.1M. In order to maintain excess alkali at all times, the original KOH concentration was set at 0.105N. This also fixed the ionic strength of the solutions at 0.105.

Care was taken to avoid the formation of carbonates through carbon dioxide absorption. All solutions were made from carbon dioxide-free water prepared by boiling and passing nitrogen through distilled water. During use and storage, care was taken to prevent reabsorption of carbon dioxide.

Two stock solutions were used in the experiments: 1M KOH (carbonate free) and 0.3M boric acid. Both were reagent-grade chemicals. The carbohydrate solutions were made up in 25- or 50-ml. quantities with concentrations near 0.3M. The boric acid and carbohydrate solutions were used to give the desired amounts of B_o and D_o in each solution. The KOH solution was used to give the needed excess of alkali. The additions were made volumetrically, using semimicro burets accurate to 0.003 ml. An error analysis showed that the instrumental error was the limiting factor in the experimental precision.

MEASUREMENT OF REFRACTIVE INDEX AND OPTICAL ROTATION

The refractive index measurements were made using a Bausch & Lomb dipping refractometer. The range of refractive index covered in this study was small

enough so that only the A-prism of the refractometer was needed. White light was used as a source, which corresponds to readings at the sodium D wavelength through internal compensation in the instrument. The temperature for all measurements was held at 30.00 ± 0.02°C. by use of a circulating water bath.

The dipping refractometer makes use of a series of tables to convert the direct instrument reading to refractive index values. However, for the present study the direct refractometer reading was found to be satisfactory. Therefore, the results were recorded in terms of R, the actual scale reading of the dipping refractometer. The refractometer was standardized to give an R_o value of 11.70 for distilled water at 30°C. which corresponds to a refractive index of 1.33197. An average of six readings was taken to obtain results with an estimated precision of ± 0.02 scale units, or ± 0.00001 expressed as refractive index.

As previously mentioned, there was some fear that carbon dioxide absorption would be a problem, but the following test showed it was not. It was found that the addition of approximately 0.05 ml. of a 0.1M sodium carbonate solution caused a definite change in the refractometer reading. The refractive index of a 0.5M alkali solution (carbonate free) was then followed over an extended period of time, with the liquid surface exposed to the air. Over a three-hour period, no change in refractive index was noted, showing negligible carbon dioxide absorption. However, in order to be doubly sure, in all stages of the study the solutions were kept in covered containers that had been flushed with nitrogen. The only exposure to air came in transfers between containers, and this was of short duration.

The optical rotation measurements were made on a Zeiss Winkel polarimeter, using the mercury wavelengths of 436 mμ and 546 mμ. Jacketed polarimeter tubes (2-decimeter length) were used, with the temperature held at 30.00 ± 0.02°C. An

average of six readings was used for the optical rotation of a solution. It was found that the values measured at the mercury green line (546 mμ) which falls in the region of greatest eye sensitivity were accurate to ± 0.005°. The decreasing sensitivity of the eye was evident in readings made using the mercury blue line (436 mμ), as the precision dropped to ± 0.01°. The drop in precision with the mercury blue line was somewhat compensated for by the greater amount of optical rotation at 436 mμ than at 546 mμ.

The following procedure was used in making all tests. A dry, 10-ml. volumetric flask was flushed with nitrogen. The desired amounts of boric acid, carbohydrate, and KOH were added in order to the volumetric flask. After the KOH addition, the solution was rapidly diluted to volume, followed by a nitrogen flush before mixing. The solution was then transferred to a special beaker for use in the refractometer. This beaker had been previously nitrogen flushed and was again flushed after the solution was added. The covered beaker was then placed in the constant-temperature bath to await use. For the refractive index reading, the refractometer prism was inserted into the solution through a rubber dam gasket which sealed the solution from the air during the reading. At least ten minutes were allowed for temperature equilibrium before readings were taken.

In cases where the optical rotation was also determined, the solutions from the refractometer test were sealed under nitrogen and placed in the refrigerator until used. Before the optical rotation readings were determined, at least fifteen minutes were allowed for the temperature to reach equilibrium.

The amount of data points depended to some degree on the amount of model compound available, but, in all cases, enough data were obtained to satisfy the experimental requirements.

RECOVERY OF MODEL COMPOUNDS

In the recovery procedure, the alkaline borate solution was first neutralized with dilute sulfuric acid and then mixed with a large excess of 95% ethanol to precipitate much of the salts. The clear ethanol solution was concentrated to a sirup which was dissolved in absolute ethanol to precipitate additional salts. The ethanol filtrate was concentrated to a sirup and dissolved in an appropriate solvent for crystallization. While the recovery procedure served its purpose in this study, it left something to be desired as the net amount recovered never exceeded 50%.

It was of interest to note that upon dissolving the ethanol precipitate in water, a basic solution was obtained that could be reprocessed to give additional model compound. It appears that some of the borate complex precipitated from ethanol and, when it was redissolved in water, dissociated through the complex equilibria into the free carbohydrate and the un-ionized boric acid. This accounts for both the alkalinity of the aqueous solution and the additional amount of recoverable model compound.

EXPERIMENTAL RESULTS

BORATE-MODEL COMPOUND RESULTS

The determination of the several constants for use in working Equations (44) and (45) is given in detail in Appendix II for all the model compounds. The several checks made on the experimental results are also given in the same appendix. The numerical values of the experimental data points are tabulated in Appendix III.

Methyl-α-D-galactopyranoside

The constants for the refractive index and optical rotation measurements are summarized in Table III.

TABLE III

REFRACTIVE INDEX AND OPTICAL ROTATION CONSTANTS FOR METHYL-α-D-GALACTOPYRANOSIDE

Constant	R	α_{436}	α_{546}
a	67.8	146.2	88.5
c	104.4	155.8	94.9
d	165.3	292.4	177.0

Using the constants given in Table III, the working equations were calculated from Equations (44) and (45).

$$\Delta R = 4.9[BD] + 15.0[BD_2] \quad (56)$$

$$\Delta\alpha_{436} = 9.6[BD] \quad (57)$$

$$\Delta\alpha_{546} = 6.4[BD] \quad (58).$$

These equations show that the optical rotation values are affected only by the BD complex, while the refractive index values are mainly affected by the BD_2 complex.

Figures 8 and 9 give the values of ΔR and $\Delta\alpha$ for the α-galactoside as a function of $[D_o]$. The curves represent third-power polynomials fitted by the least squares method and are defined by

$$\Delta R = 0.005 + 0.23[D_o] + 118.02[D_o]^2 - 1203.65[D_o]^3 \quad (59),$$

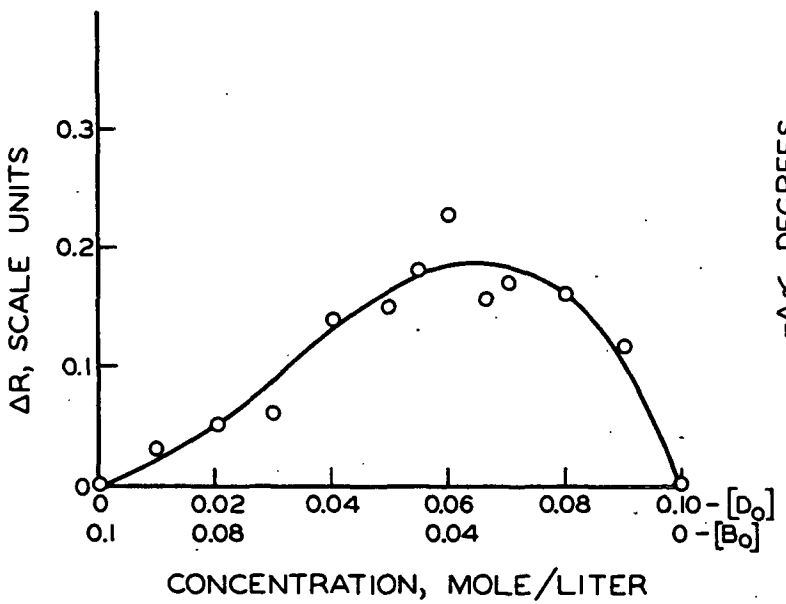


Figure 8. Variation of ΔR with Molar Ratio of Methyl-α-D-galactopyranoside to Borate

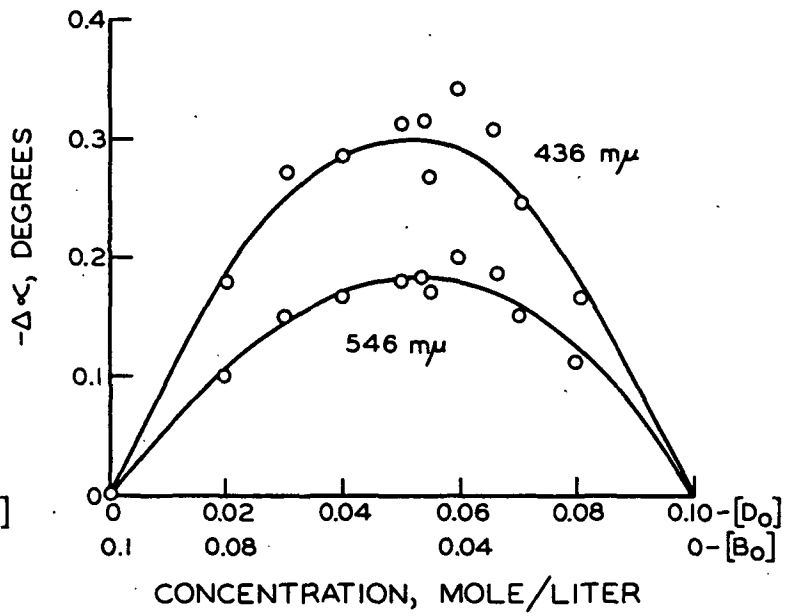


Figure 9. Variation of $\Delta\alpha$ with Molar Ratio of Methyl-α-D-galactopyranoside to Borate

$$\Delta\alpha_{436} = 0.004 - 12.24[D_o] + 119.12[D_o]^2 + 38.89[D_o]^3 \quad (60),$$

$$\Delta\alpha_{546} = 0.002 - 6.35[D_o] + 44.23[D_o]^2 + 194.85[D_o]^3 \quad (61).$$

The 95% confidence limits at a $[\underline{D}_o]$ of 0.05 mole/liter for the $\Delta\underline{R}$ and $\Delta\alpha$ values are $\pm$ 0.02-0.03. These are also the error limits expected from a consideration of the experimental errors in the measurement of $\underline{R}$ and α.

Using the values from Equations (59) and (61) to calculate [BD] and $[BD_2]$, the concentrations given in Fig. 10 and 11 were obtained. Once [BD] and $[BD_2]$ were known, [D] and [B] were calculated from the mass balance equations and are given in Fig. 12 and 13. The [BD] values were calculated using the $\Delta\alpha_{546}$ data since there was less scatter in these points than in the $\Delta\alpha_{436}$ data. The [BD] values were substituted into Equation (56), and the $[BD_2]$ values were calculated directly. From a consideration of the experimental error, these values are accurate to $\pm$ 0.002 mole/liter.

Once the concentrations of all the free components were known, the stability constants were calculated, using the concentration data at $[\underline{D}_o]$ values of 0.05 and 0.06 mole/liter. The stability constants (see p. 43) are given in Table IV.

TABLE IV

STABILITY CONSTANTS FOR THE METHYL-α-D-GALACTOPYRANOSIDE-BORATE COMPLEX

$[\underline{D}_o]$, mole/liter	β_1	β_2	$\underline{K}_2$	$\underline{K}_T$
0.05	80	490	--	--
0.06	120	310	--	--
Average	100	400	4	4×10^4

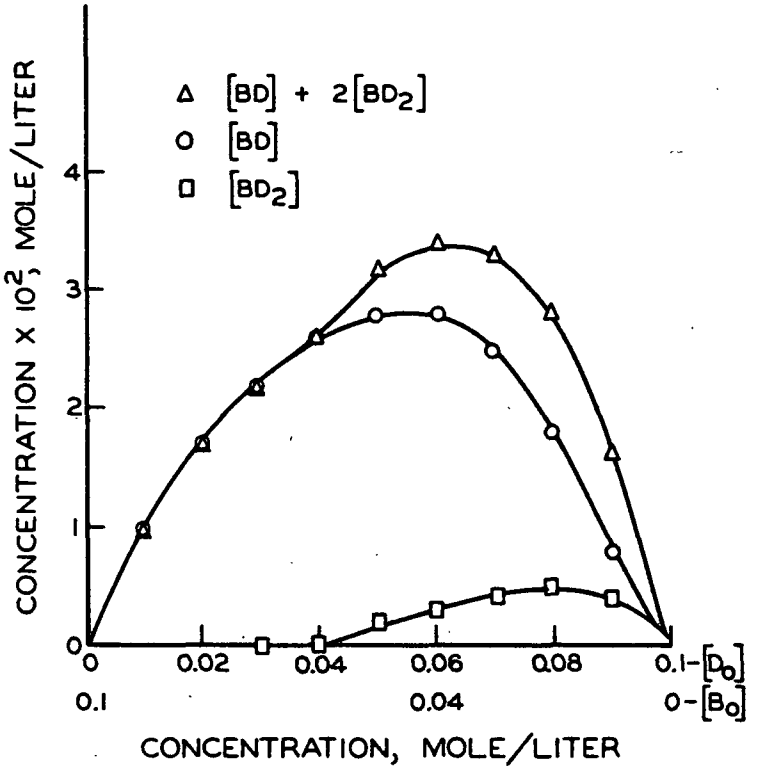


Figure 10. Concentration of the Borate Complexes as a Function of the Molar Ratio of Methyl-α-D-galactopyranoside to Borate

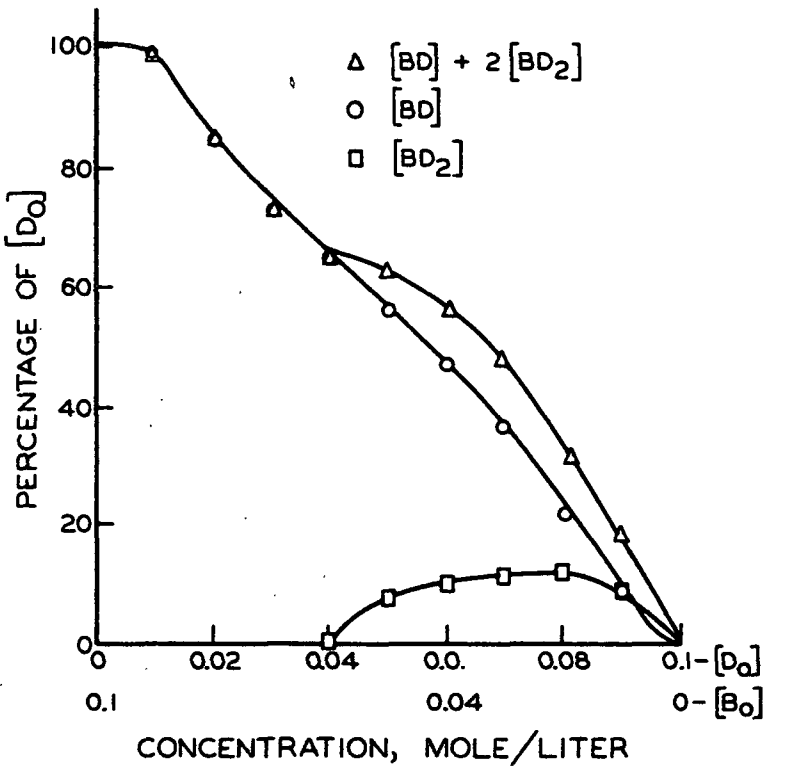


Figure 11. Percentage of [D_o] in Each Borate Complex as a Function of the Molar Ratio of Methyl-α-D-galactopyranoside to Borate

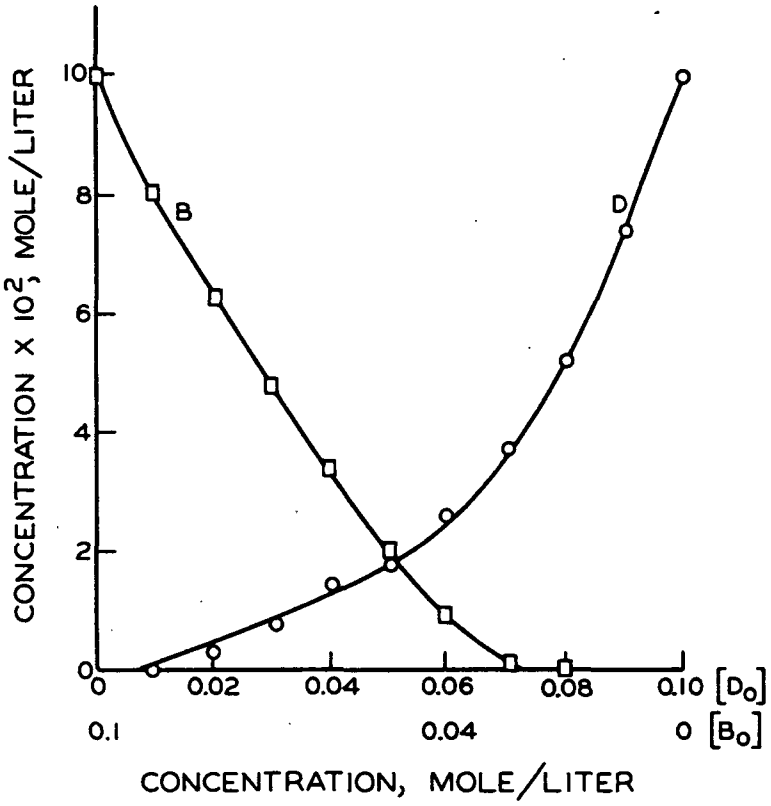


Figure 12. Concentration of D and B as a Function of the Molar Ratio of Methyl-α-D-galactopyranoside to Borate

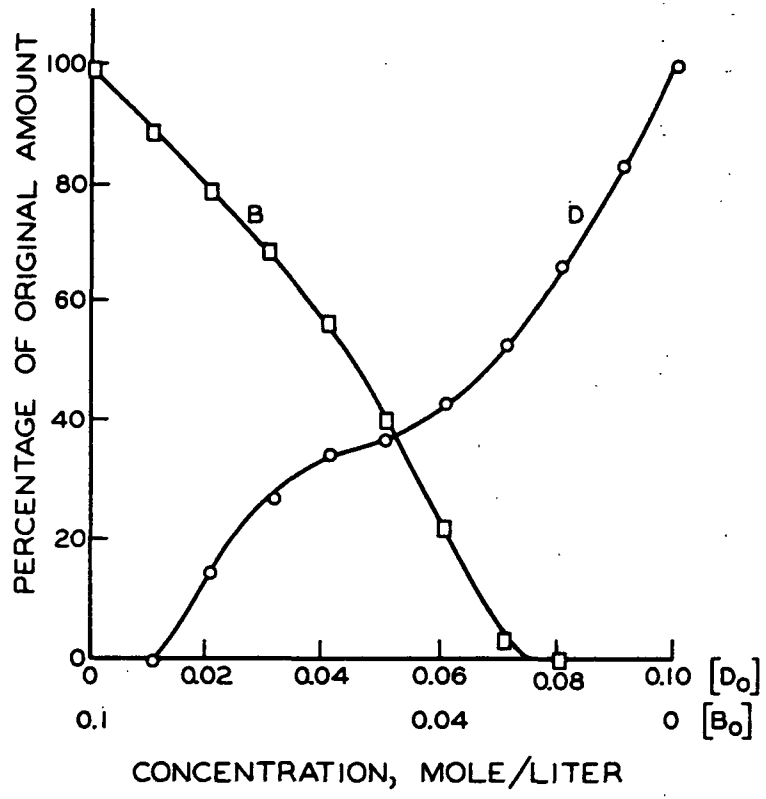


Figure 13. Percentage of [D] and [B] as a Function of the Molar Ratio of Methyl-α-D-galactopyranoside to Borate

Methyl-α-D-mannopyranoside

The second model compound studied was the methyl-α-D-mannopyranoside. The refractive index and optical rotation constants are summarized in Table V.

TABLE V

SUMMARY OF REFRACTIVE INDEX AND OPTICAL ROTATION CONSTANTS FOR METHYL-α-D-MANNOPYRANOSIDE

Constant	R	α_{436}	α_{546}
a	66.4	59.8	36.3
c	111.0	41.8	25.9
d	166.4	106.9	64.6

Using the constants given in Table V, the following working equations were obtained.

$$\Delta R = 11.0[BD_2] - 0.3[BD] \quad (62),$$

$$\Delta\alpha_{436} = 18.0[BD] + 13.0[BD_2] \quad (63),$$

$$\Delta\alpha_{546} = 10.4[BD] + 10.0[BD_2] \quad (64).$$

From these equations it can be seen that the $\Delta\alpha$ values are affected by both BD and BD_2. On the other hand, the ΔR values are almost entirely due to BD_2. However, the reliability of the BD_2 coefficient in Equation (62) is open to doubt due to the scatter obtained in the determination of the d constant for refractive index (see Appendix II).

The experimental values for ΔR and $\Delta\alpha$ are shown in Fig. 14 and 15. The polynomials for the curves have the form:

$$\Delta R = 0.0007 - 13.38[D_o] + 804.04[D_o]^2 + 12,425.87[D_o]^3 + 57,226.00[D_o]^4 \quad (65),$$

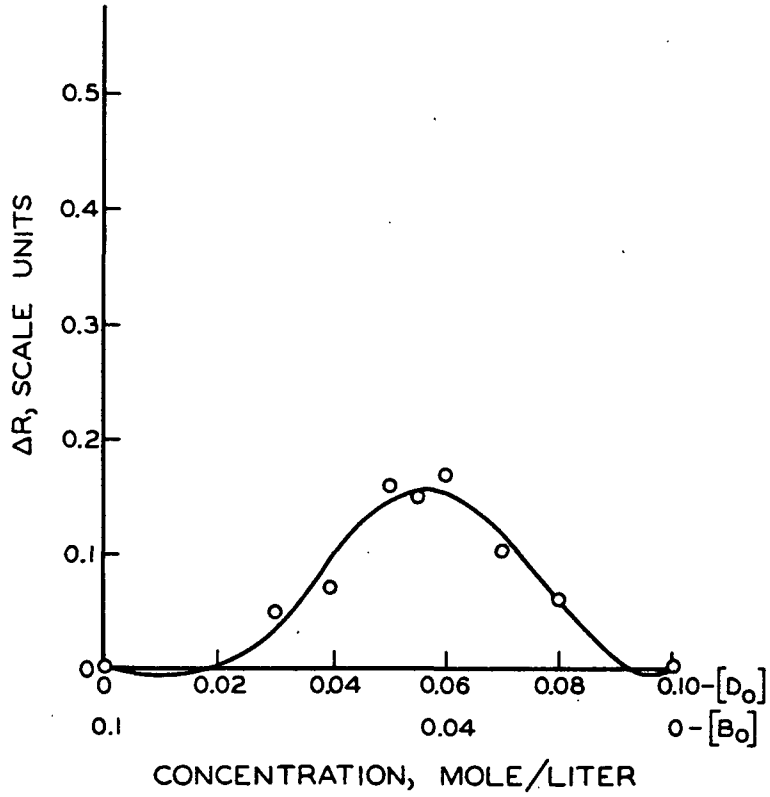


Figure 14. Variation of $\Delta\underline{R}$ with the Molar Ratio of Methyl-α-$\overline{D}$-manno-pyranoside to Borate

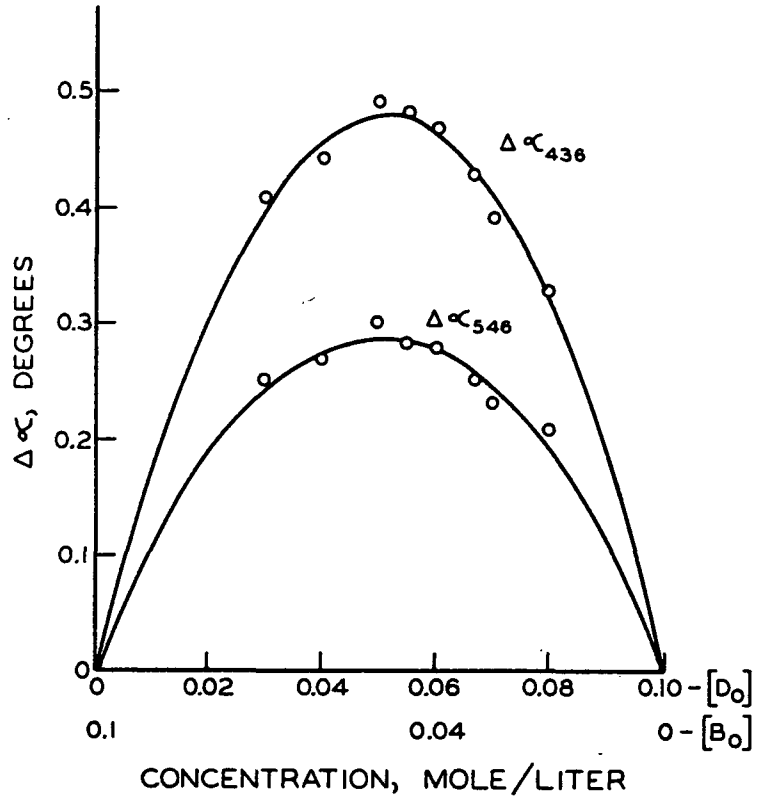


Figure 15. Variation of $\Delta\alpha$ with the Molar Ratio of Methyl-α-D-manno-pyranoside to Borate

$$\Delta\alpha_{436} = 0.001 + 18.60[D_o] - 174.36[D_o]^2 - 114.86[D_o]^3 \quad (66),$$

and $$\Delta\alpha_{546} = 0.001 + 11.46[D_o] - 11.83[D_o]^2 - 4.71[D_o]^3 \quad (67).$$

The fourth-power polynomial used to fit the $\Delta\underline{R}$ data deviated at the ends of the curve, and, as was mentioned, the BD_2 coefficient in the $\Delta\underline{R}$ working equation was not too reliable. Therefore, while the $\Delta\underline{R}$ curve showed the relative manner in which $[BD_2]$ changes, it could not be used to calculate the absolute $[BD_2]$ values. The concentrations of BD and BD_2 were calculated from the optical rotation data, with the results shown in Fig. 16 and 17. The BD and BD_2 concentrations were then used to calculate [D] and [B], and these values are given in Fig. 18 and 19. The 95% confidence limits at a $[\underline{D}_o]$ value of 0.05 mole/liter for the $\Delta\alpha$ values were $\pm$ 0.015°, which again are approximately the same as the variation expected from experimental error. It should be emphasized that the valid area in these curves lies between $[\underline{D}_o]$ values of 0.035 to 0.075 mole/liter. Results outside of these limits represent extensions of polynomial values beyond the main body of experimental points and must be considered with some caution.

Using the concentration values shown in the graphs, the stability constants for the α-mannoside-borate system were calculated. These values are given in Table VI.

TABLE VI

STABILITY CONSTANTS FOR THE METHYL-α-D-MANNOPYRANOSIDE-BORATE SYSTEM

$[\underline{D}_o]$, mole/liter	β_1	β_2	$\underline{K}_2$	$\underline{K}_T$
0.05	61	785	--	--
0.06	61	456	--	--
Average	60	600	10	4×10^4

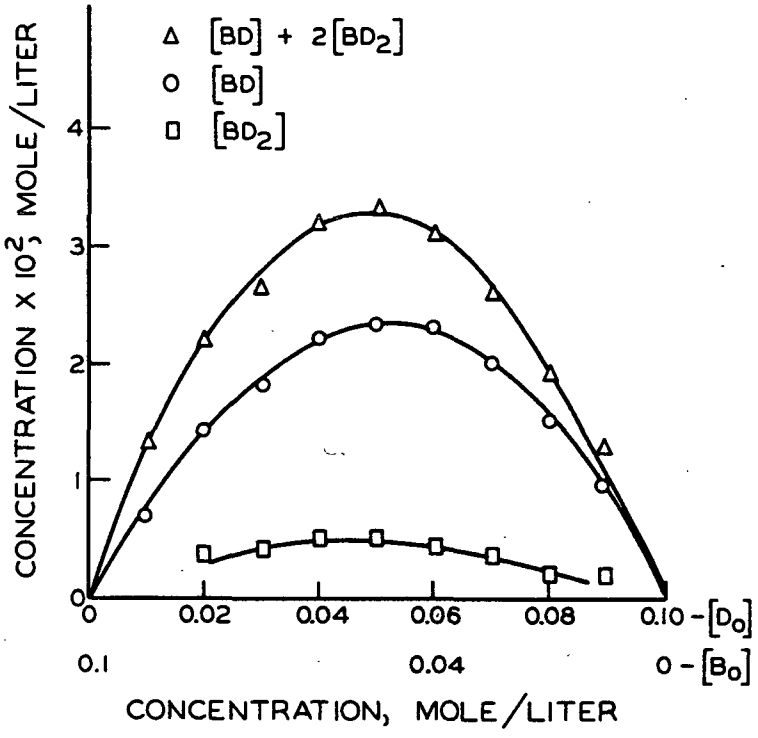


Figure 16. Variation of the Borate Complexes as a Function of the Molar Ratio of Methyl-α-D-mannopyranoside to Borate

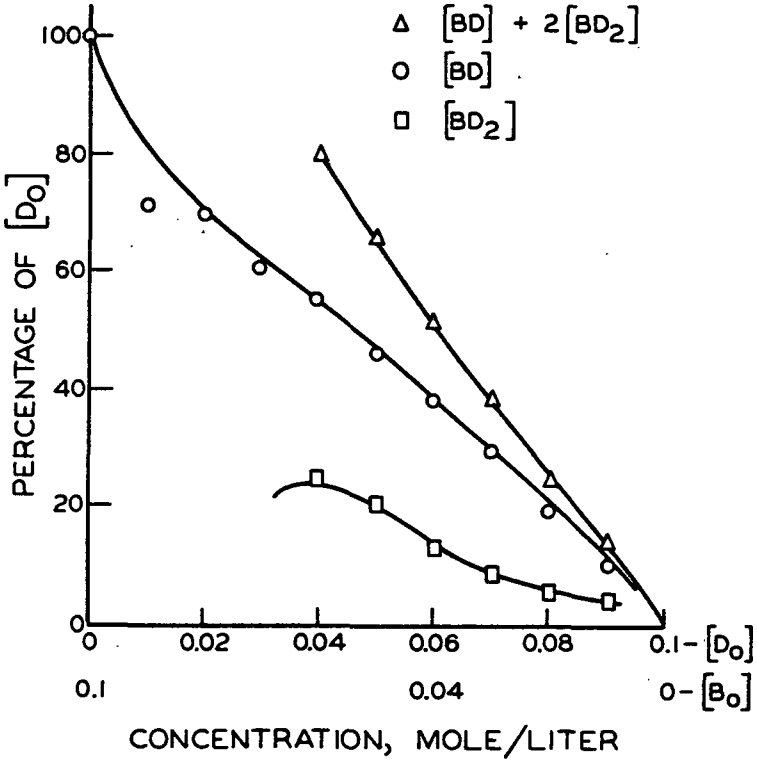


Figure 17. Percentage of $[D_o]$ in Each Complex as a Function of the Molar Ratio of Methyl-α-D-mannopyranoside to Borate

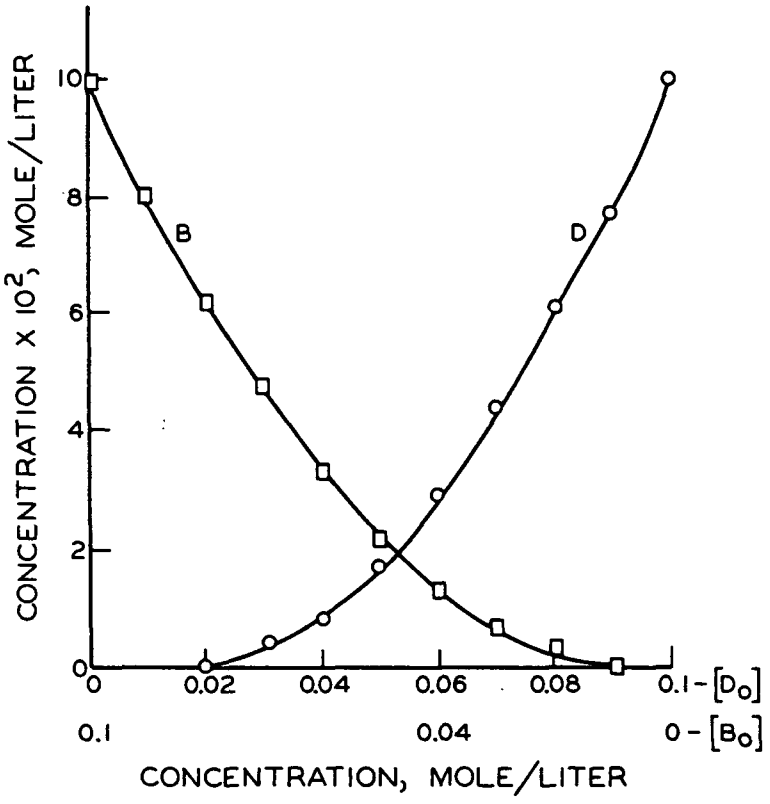


Figure 18. Concentration of D and B as a Function of the Molar Ratio of Methyl-α -D-mannopyranoside to Borate

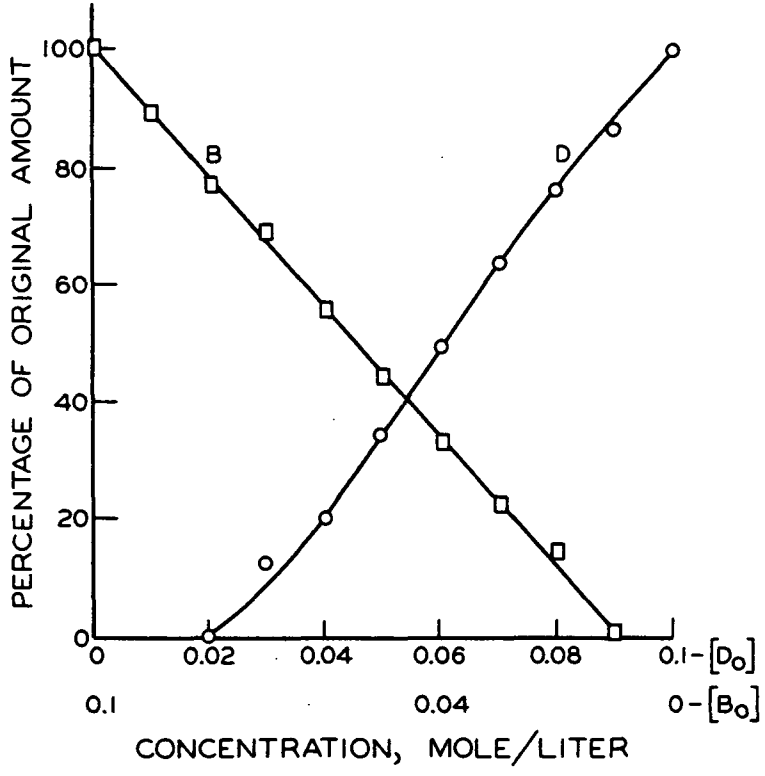


Figure 19. Percentage of [D] and [B] as a Function of the Molar Ratio of Methyl-α-D-mannopyranoside to Borate

The results are quite similar to those for the α-galactoside with a tendency toward more of the BD_2 complex. This will be discussed in greater detail later in this report.

Methyl-4-O-methyl-α-D-mannopyranoside

The third model compound studied was the methyl-4-O-methyl-α-mannoside. The refractive index and optical rotation constants are summarized in Table VII.

TABLE VII

REFRACTIVE INDEX AND OPTICAL ROTATION CONSTANTS FOR METHYL-4-O-METHYL-α-D-MANNOPYRANOSIDE

Constant	R	α_{436}	α_{546}
a	69.8	66.4	40.3
c	44.7	56.4	34.1
d	162.4	113.4	69.6

Using the above constants, the following working equations were obtained.

$$\Delta R = 69.8[BD] + 21.8[BD_2] \quad (68),$$

$$\Delta\alpha_{436} = 10.0[BD] + 20.0[BD_2] \quad (69),$$

$$\Delta\alpha_{546} = 6.2[BD] + 11.0[BD_2] \quad (70).$$

From these equations it is apparent that both the optical rotation and the refractive index measurements are affected appreciably by both the BD and BD_2 complexes. However, the [BD] coefficient in the ΔR working equation appears unusually large; a check on this coefficient by the ratio test will be given in Appendix II.

Figures 20 and 21 give ΔR and $\Delta\alpha$ as a function of $[D_o]$. The curves are defined by the following equations.

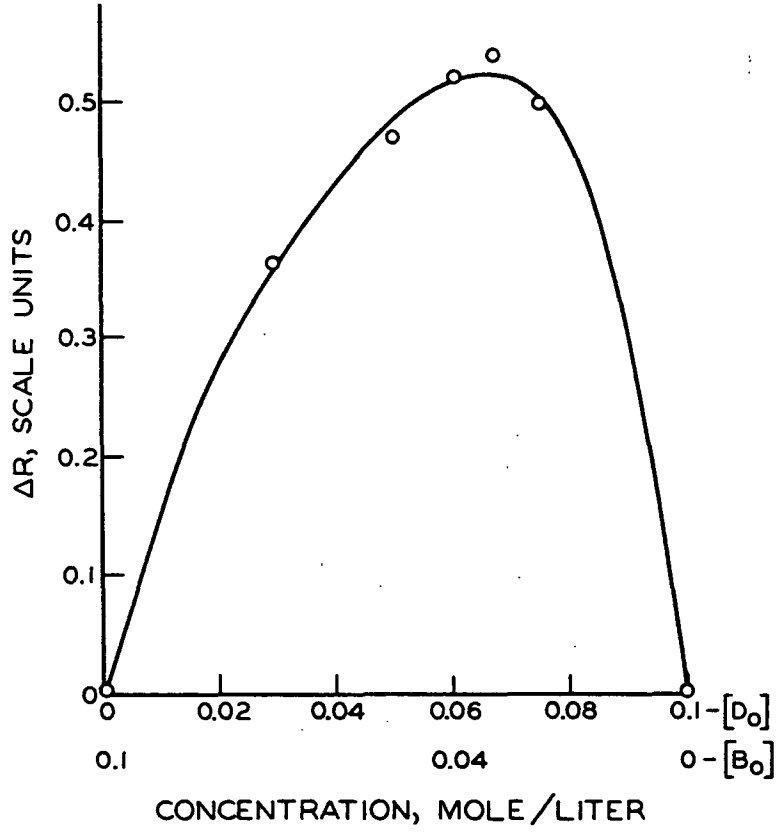


Figure 20. Variation of ΔR with the Molar Ratio of Methyl-4-O-Methyl-α-D-mannopyranoside to Borate

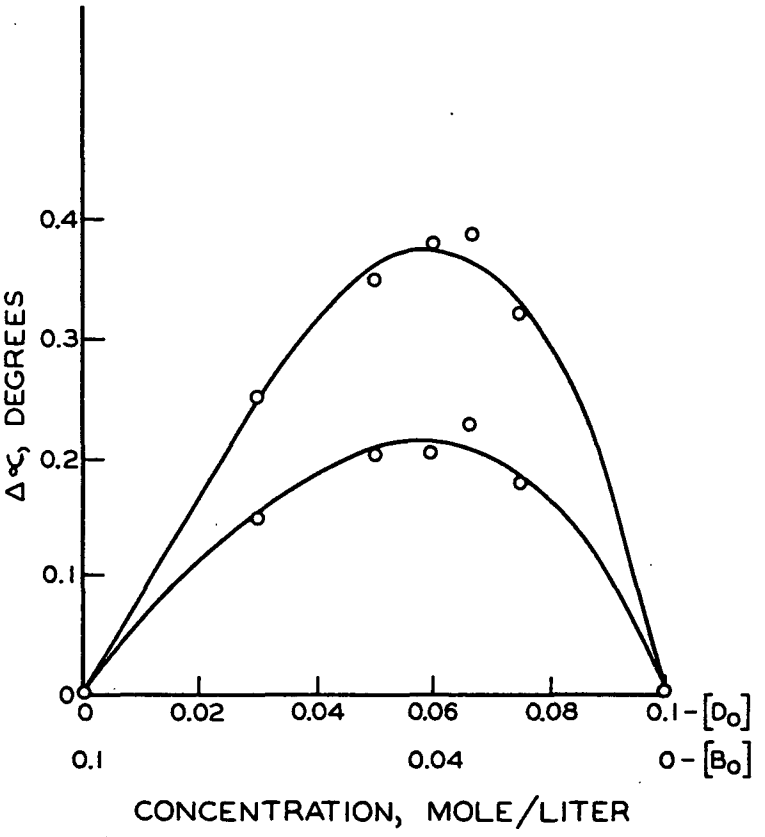


Figure 21. Variation of $\Delta\alpha$ with the Molar Ratio of Methyl-4-O-methyl-α-D-mannopyranoside to Borate

$$\Delta R = 0.0002 + 21.07[D_o] - 488.40[D_o]^2 + 7549.46[D_o]^3 - 47{,}727.65[D_o]^4 \quad (71),$$

$$\Delta\alpha_{436} = 0.0002 + 7.73[D_o] + 56.89[D_o]^2 - 1342.85[D_o]^3 \quad (72),$$

$$\Delta\alpha_{546} = 0.0007 + 4.87[D_o] + 18.12[D_o]^2 - 668.60[D_o]^3 \quad (73).$$

As has been mentioned, the working equation for ΔR is open to some doubt. Therefore, the BD and BD_2 concentrations were calculated from the optical rotation data, with the results given in Fig. 22 and 23. The [D] and [B] values were also calculated and are shown in Fig. 24 and 25.

The 95% confidence limits for the $\Delta\alpha$ values at a $[D_o]$ value of 0.05 mole/liter were about $\pm$ 0.03, or roughly equivalent to the experimental error. This can give rise to maximum variation in [BD] of $\pm$ 0.04 mole/liter and in $[BD_2]$ of $\pm$ 0.03 mole/liter. These limits are large, with a large change in concentration occurring from a small change in $\Delta\alpha$. It now becomes apparent that much was gained by using a curve over a range of concentrations as opposed to calculating an individual point. While the wide limits might place doubt on an individual point, when the point is part of a sequence of data points, its reliability increases. These wide error limits seem somewhat strange as the 4-O-methyl-α-mannoside system gave the most consistent pattern of all four model compounds. It leads to a feeling that the data are better than the error analysis indicates.

Once the concentrations of the components of the 4-O-methyl-α-mannoside–borate system were known, the stability constants were calculated and are given in Table VIII.

In general, the total amount of complex increased, with most of the increase due to the BD_2 complex.

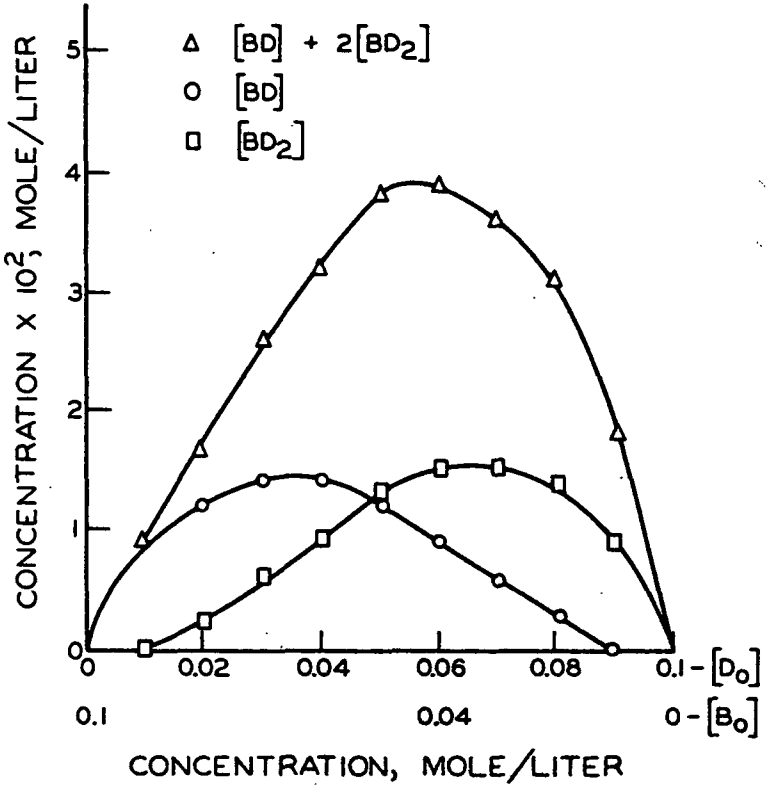


Figure 22. Concentration of the Borate Complex as a Function of the Molar Ratio of Methyl-4-O-methyl-α-D-mannopyranoside to Borate

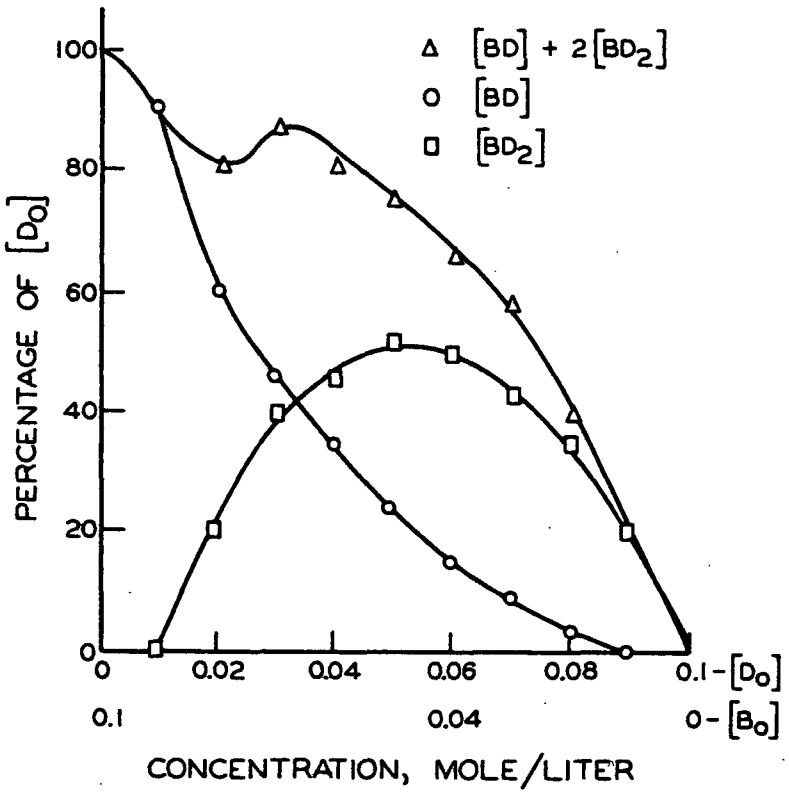


Figure 23. Percentage of [D$_o$] in Each Borate Complex as a Function of the Molar Ratio of Methyl-4-O-methyl-α-D-mannopyranoside to Borate

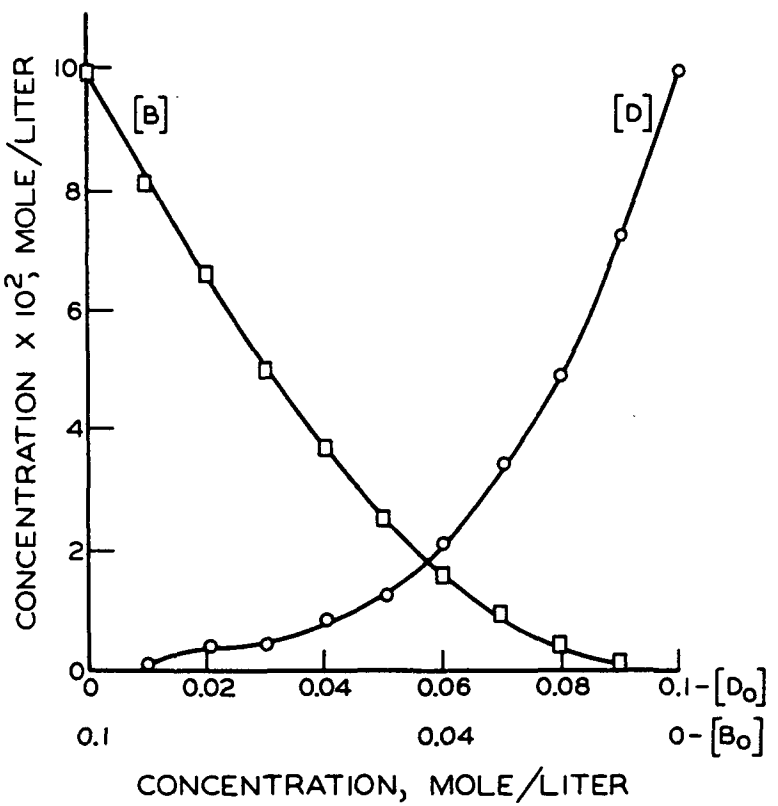


Figure 24. Concentration of D and B as a Function of the Molar Ratio of Methyl-4-O-methyl-α-D-mannopyranoside to Borate

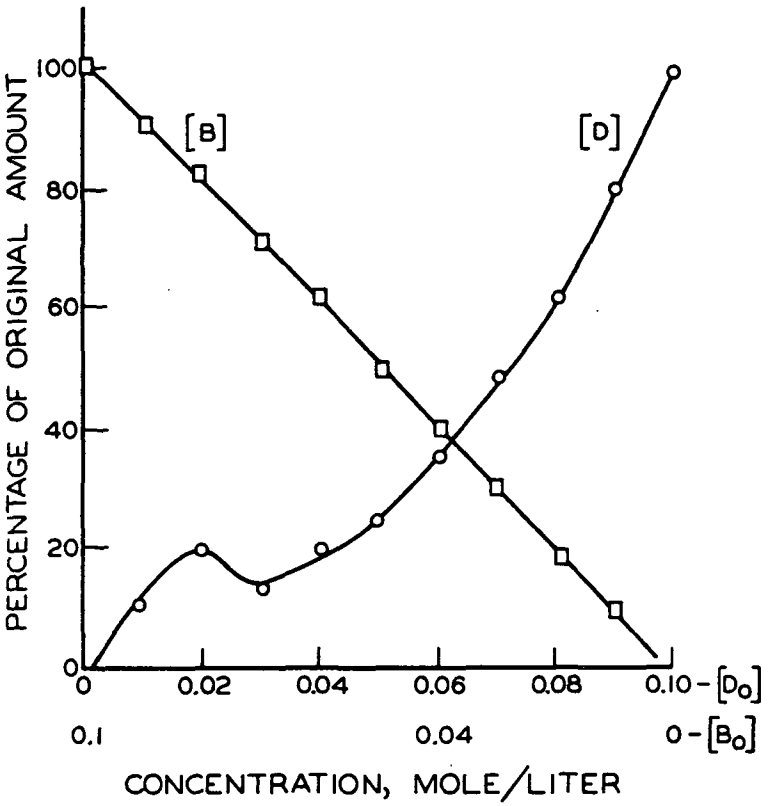


Figure 25. Percentage of [D] and [B] as a Function of the Molar Ratio of Methyl-4-O-methyl-α-D-mannopyranoside to Borate

TABLE VIII

STABILITY CONSTANTS FOR THE METHYL-4-O-METHYL-α-D-MANNOPYRANOSIDE–BORATE SYSTEM

$[D_o]$, mole/liter	β_1	β_2	K_2	K_T
0.05	40	3600	--	--
0.06	27	2120	--	--
Average	30	3000	100	1×10^5

Methyl-4-O-methyl-β-D-mannopyranoside

The fourth model compound studied was the methyl-4-O-methyl-β-mannoside. The optical rotation and refractive index constants are summarized in Table IX.

TABLE IX

REFRACTIVE INDEX AND OPTICAL ROTATION CONSTANTS FOR THE METHYL-4-O-METHYL-β-D-MANNOPYRANOSIDE–BORATE SYSTEM

Constant	R	α_{436}	α_{546}
a	70.6	-45.8	-28.2
c	80.2	-46.4	-30.3
d	--	-103.6	-63.1

One of the usual constants is missing, that is, that resulting from the $[BD_2]$ versus R data. Due to experimental difficulties, this relation was not established before all of the 4-O-methyl-β-mannoside had been used. As will be discussed, the $[BD_2]$ coefficient for the ΔR working equation was determined from the ΔR data points.

Using the constants in Table IX, the following working equations were obtained:

$$\Delta R = 35.0[BD] + \underline{X}[BD_2] \quad (74),$$

where $\underline{X}$ represents the unknown $[BD_2]$ coefficient,

$$\Delta\alpha_{436} = 0.6[BD] + 12.0[BD_2] \quad (75),$$

and

$$\Delta\alpha_{546} = 2.1[BD] + 6.7[BD_2] \quad (76).$$

As will be shown later, [BD] has a maximum value of approximately 0.005 mole/liter. Hence, the [BD] term in Equation (75) makes no significant contribution, and the equation can be rewritten as

$$\Delta\alpha_{436} = 12.0[BD_2] \quad (77).$$

Similarly, the [BD] term in Equation (76) is of minor significance, and, in general, the optical rotation data follow only the BD_2 complex. This is due primarily to the small difference between the $\underline{a}$ and $\underline{c}$ constants for the optical rotation measurements.

The changes in $\Delta\underline{R}$ and $\Delta\alpha$ with changing $[\underline{D}_o]$ are shown in Fig. 26 and 27. The $\Delta\underline{R}$ curve does not contain enough points to warrant the calculation of a polynomial. The solid curved line is an estimation of how the curve should look, using the 4-$\underline{O}$-methyl-α-mannoside system as a model. The polynomials for the $\Delta\alpha$ curves are defined by the following equations.

$$\Delta\alpha_{436} = 0.001 - 2.48[D_o] + 181.15[D_o]^2 - 1566.61[D_o]^3 \quad (78)$$

$$\Delta\alpha_{546} = 0.0001 - 1.70[D_o] + 102.04[D_o]^2 - 850.50[D_o]^3 \quad (79).$$

Because the coefficients of the $\Delta\alpha$ equations were based on relatively few data points, the $\Delta\alpha$ data points were used with the ratio test to adjust the coefficients. Since the [BD] contribution will be small, only the $[BD_2]$ coefficient was tested.

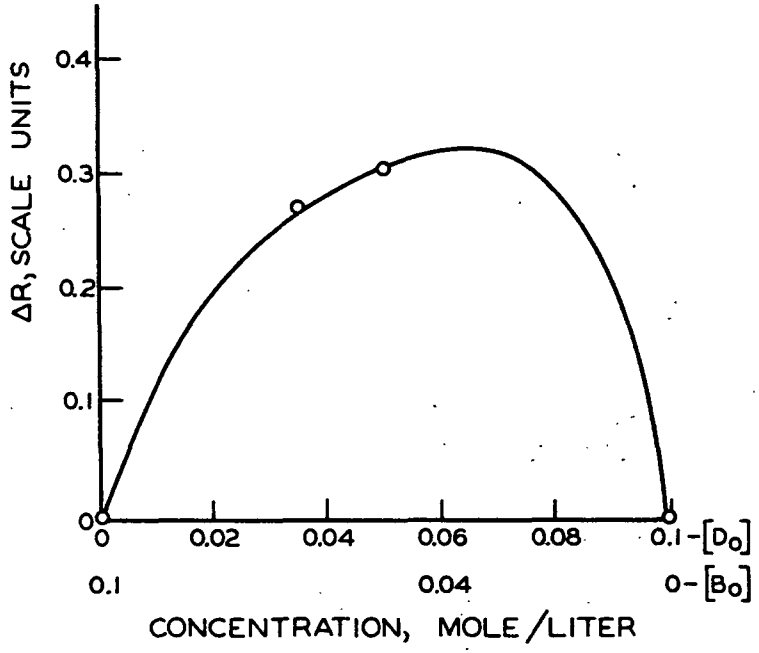


Figure 26. Variation of ΔR with the Molar Ratio of Methyl-4-O-methyl-β-D-manno-pyranoside to Borate

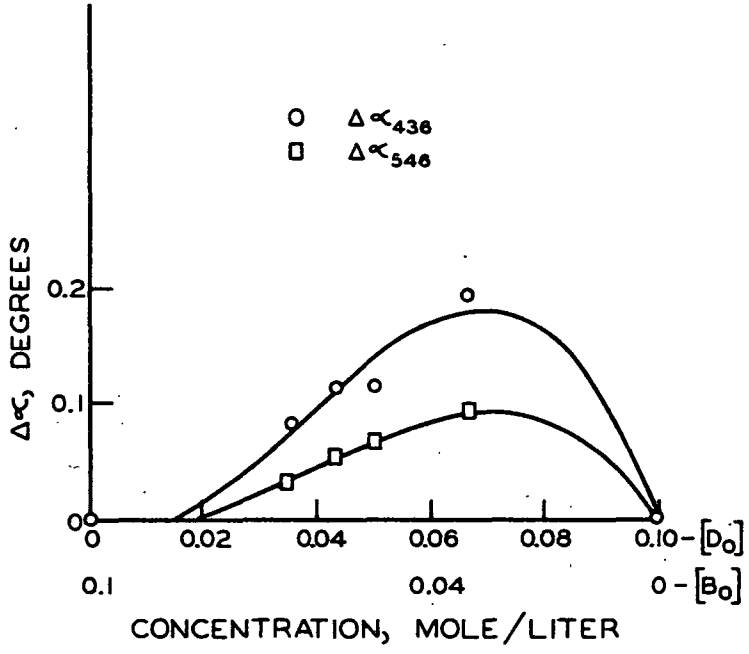


Figure 27. Variation of Δα with the Molar Ratio of Methyl-4-O-methyl-β-D-manno-pyranoside to Borate

If no [BD] is present, the $\Delta\alpha$ equations give

$$\Delta\alpha_{436}/\Delta\alpha_{546} = 12.0/6.7 = 1.8 \quad (80),$$

while the data points at $[\underline{D}_o]$ equal to 0.09 mole/liter give

$$\Delta\alpha_{436}/\Delta\alpha_{546} = 1.96 \quad (81).$$

By a slight adjustment of the $[BD_2]$ coefficients to 12.4 and 6.3, respectively, the ratio tests check almost exactly. This, in effect, brings the $\Delta\alpha$ data into the calculation of the coefficients. The new equations are:

$$\Delta\alpha_{436} = 12.4[BD_2] \quad (82),$$

and

$$\Delta\alpha_{546} = 2.1[BD] + 6.3[BD_2] \quad (83).$$

This minor adjustment actually makes little if any difference in the $[BD_2]$ values. However, it serves to bring the [BD] values close to zero, instead of being slightly negative.

Using Equation (82), the $[BD_2]$ values were calculated and are shown in Fig. 28 and 29. When the $[BD_2]$ values were substituted into Equation (83), the resulting [BD] values were all near zero. From a consideration of experimental error, the variance in $[BD_2]$ was found to be $\pm$ 0.003 mole/liter. The $[BD_2]$ values were also calculated from the $\Delta\alpha_{546}$ data assuming no [BD] and agreed to 0.001 mole/liter with the $\Delta\alpha_{436}$ values.

Because small amounts of BD would not affect the $\Delta\alpha$ equations, the $\Delta\underline{R}$ equation had to be used to determine the BD concentration. Before this could be done, the $[BD_2]$ coefficient of the $\Delta\underline{R}$ equation had to be estimated by using the $\Delta\underline{R}$ data from regions where the amount of BD could be assumed negligible.

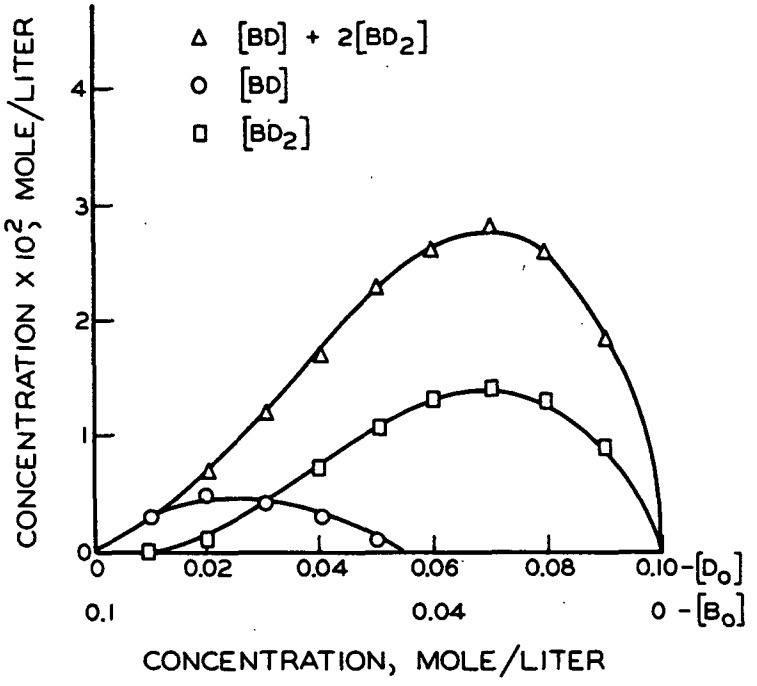


Figure 28. Concentration of the Borate Complexes as a Function of the Molar Ratio of Methyl-4-O-methyl-β-D-mannopyranoside to Borate

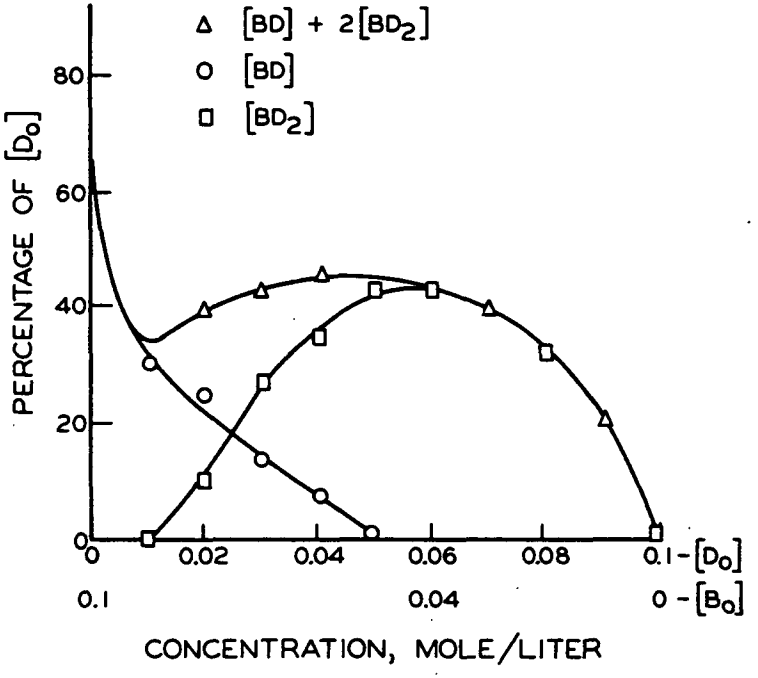


Figure 29. Percentage of [D_o] in Each Borate Complex as a Function of the Molar Ratio of Methyl-4-O-methyl-β-D-mannopyranoside to Borate

With $\Delta\underline{R}$ and $[BD_2]$ known and [BD] negligible, $\underline{X}$ can be calculated directly from Equation (74), which on rearrangement gives

$$X = \Delta R/[BD_2] \qquad (84).$$

The results of the calculations are given in Table X.

TABLE X

ESTIMATION OF THE $[BD_2]$ COEFFICIENT IN THE $\Delta\underline{R}$ EQUATION

$[\underline{D}_o]$, mole/liter	$\Delta\underline{R}$	$[BD_2]$	$\underline{X}_{calcd.}$
0.06	0.32	0.013	25
0.07	0.32	0.014	24
0.08	0.29	0.013	22
0.09	0.21	0.009	23

From the above values, an average value of $\underline{X} = 23.0$ is obtained, which was then checked by the ratio test.

If no BD is present, the working equations give

$$\Delta\alpha_{436}/\Delta\underline{R} = 12.4/23 = 0.54 \quad \text{and} \quad \Delta\alpha_{546}/\Delta\underline{R} = 6.2/23 = 0.29.$$

From the data points at $[\underline{D}_o]$ equal to 0.09 mole/liter,

$$\Delta\alpha_{436}/\Delta\underline{R} = 0.53 \quad \text{and} \quad \Delta\alpha_{546}/\Delta\underline{R} = 0.26.$$

This checks rather well. However, it must be remembered that the $\Delta\underline{R}$ values were taken from the estimated curve in Fig. 26. The $\Delta\underline{R}$ working equation becomes

$$\Delta R = 35[BD] + 23[BD_2] \qquad (85).$$

Using Equation (85), the [BD] values were calculated and are given in Fig. 28 and 29. While these [BD] values were obtained using estimated coefficients, they are reasonable as they were small as required by the optical rotation results and followed the same general pattern as the other model compounds. If the error in $\underline{X}$ is taken to extremes, and it is assumed that $\underline{X}$ is zero, the [BD] values still do not go over 0.009 mole/liter. Therefore, the [BD] values are reliable and can be used in making comparisons with the other model compounds. The calculated values of [D] and [B] for the 4-O-methyl-β-mannoside system are given in Fig. 30 and 31.

With the concentration of the components of the 4-O-methyl-β-mannoside system known, the stability constants were calculated and are given in Table XI.

TABLE XI

STABILITY CONSTANTS FOR THE METHYL-4-O-METHYL-β-D-MANNOPYRANOSIDE-BORATE SYSTEM

$[D_o]$, mole/liter	β_1	β_2	K_2	K_T
0.04	2.6	--	--	--
0.05	1.0	383	--	--
0.06	--	416	--	--
Average	2.0	400	200	800

In general, the total amount of complexing dropped, primarily due to the large drop in the amount of the monocomplex BD.

DISCUSSION OF RESULTS AND CONCLUSIONS

TYPE OF COMPLEX PRESENT IN SOLUTION

The continuous variations method indicated the presence of two types of borate complexes, i.e., the monocomplex, BD, and the dicomplex, BD_2. Because

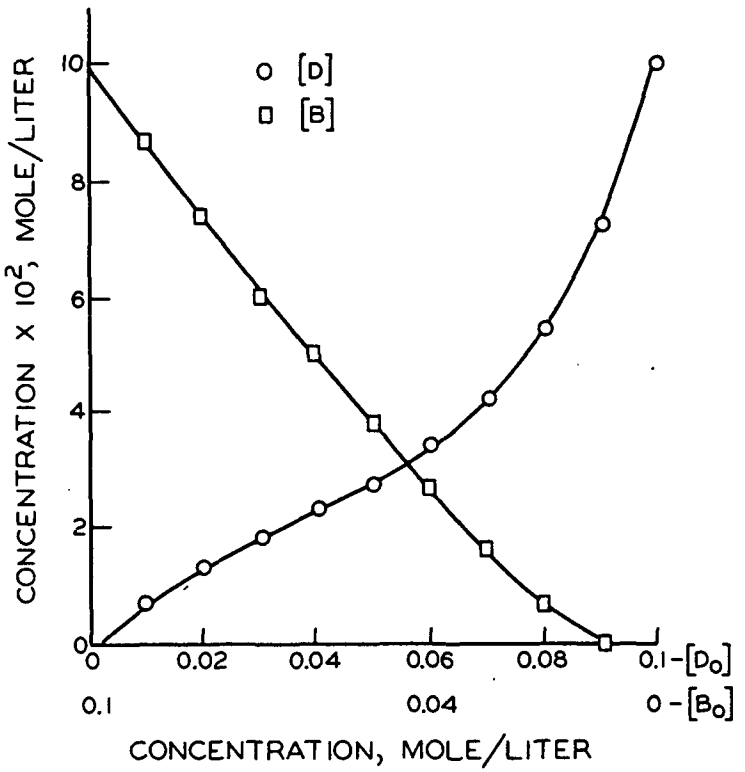


Figure 30. Concentration of D and B as a Function of the Molar Ratio of Methyl-4-O-methyl-β-D-mannopyranoside to Borate

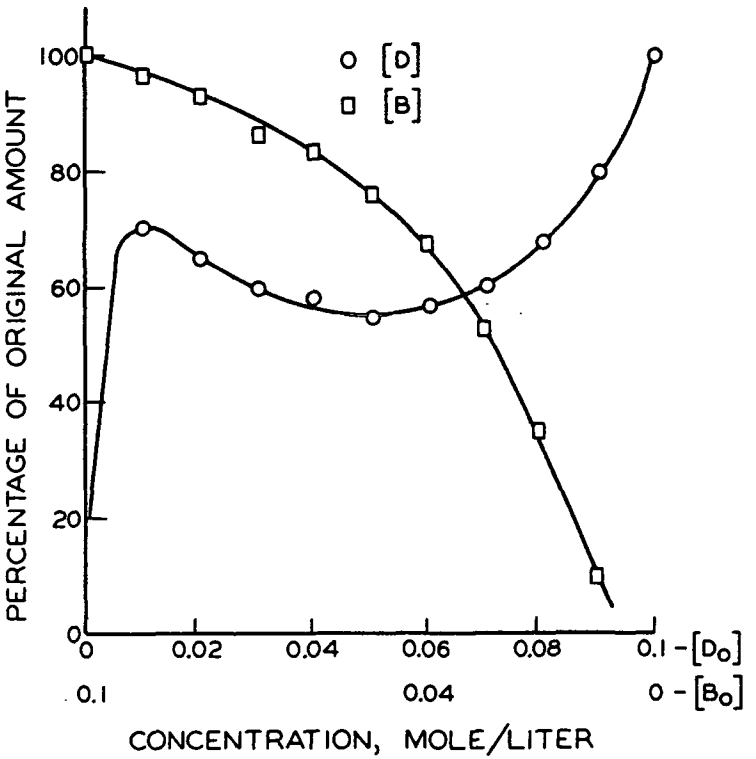


Figure 31. Percentage of [D_o] and [B] as a Function of the Molar Ratio of Methyl-4-O-methyl-β-D-mannopyranoside to Borate

two types of complexes were present, the peak in the continuous variations curves of the refractive index and optical rotation measurements was shifted toward an average value. In all cases the working equations were used to interpret the curves. For example, the working Equations (56)-(58) for the α-galactoside show that the optical rotation values are affected only by the BD complex, while the refractive index variation is mainly the result of changes in the amount of BD_2 complex. As is seen in Fig. 8 and 9, the optical rotation curve peaked near a 1:1 ratio of $[\underline{D}_o]$ to $[\underline{B}_o]$, corresponding to the BD complex, and the refractive index curve peaked near a 2:1 ratio of $[\underline{D}_o]$ to $[\underline{B}_o]$, corresponding to the BD_2 complex. These results point up the care that must be taken in interpreting continuous variations data, as different measurements can indicate different results.

The continuous variation results with the other model compounds are not as clear cut; however, in all cases the presence of the two complexes is definitely indicated. With the α-mannoside, the working Equations (62)-(64) indicate that the refractive index measurements are influenced to a greater extent by the BD_2 complex than are the optical rotation measurements. Thus, the $\Delta\underline{R}$ curve peaks more toward the 2:1 $[\underline{D}_o]$ to $[\underline{B}_o]$ ratio than the $\Delta\alpha$ curves (Fig. 14 and 15). In the case of the 4-O-methyl-α-mannoside, both refractive index and optical rotation are affected strongly by both complexes, with the BD_2 effect somewhat greater, as shown by the larger coefficient in Equations (69), (70), and (105). Hence, both the $\Delta\underline{R}$ and $\Delta\alpha$ curves (Fig. 20 and 21) peak closer to a 2:1 than to a 1:1 ratio of $[\underline{D}_o]$ to $[\underline{B}_o]$. With the 4-O-methyl-β-mannoside, the effect of the BD_2 complex dominates in the $\Delta\alpha$ curve (Fig. 27), and the optical rotation curve peaks near a 2:1 ratio of $[\underline{D}_o]$ to $[\underline{B}_o]$. Because the relative amount of BD is small with this model compound, the refractive index curve (Fig. 26) is also dominated by the BD_2 complex.

Additional proof of the presence of the complexes is provided by the many linear relations obtained as predicted on a basis of the formation of two complexes. Such procedures as the back-calculation of $[\underline{D}_o]$ also serve to confirm that no additional complexes were present.

RELATIVE COMPLEXING ABILITY OF THE MODEL COMPOUNDS

In the case of the α-galactoside, the BD complex dominated, with a peak concentration near 0.028 mole/liter at a $[\underline{D}_o]$ value of 0.055 mole/liter. Only small amounts of the BD_2 complex were obtained, with a maximum concentration of 0.003 mole/liter occurring at a $[\underline{D}_o]$ of 0.08 mole/liter. The α-mannoside gave results similar to the α-galactoside, with a slight increase in the BD_2 complex and a decrease in the BD complex. In contrast, a large change occurred with the 4-O-methyl-α-mannoside, as an increase occurred in both the BD_2 complex and in the total amount of complex formation. The BD complex was a maximum at 0.014 mole/liter at a $[\underline{D}_o]$ of 0.035 mole/liter, while the BD_2 complex peaked at 0.014 mole/liter at a $[\underline{D}_o]$ of 0.065 mole/liter. The 4-O-methyl-β-mannoside gave the least total complexing of the model compounds, but in contrast to the other compounds it was dominated by the BD_2 complex. The BD_2 complex peaked near 0.013 mole/liter at a $[\underline{D}_o]$ value of 0.070 mole/liter, while the BD complex was at a maximum near 0.004 mole/liter at a $[\underline{D}_o]$ of 0.025 mole/liter.

The results can also be viewed from the standpoint of the stability constants (Table XII) which follow the same trend as previously mentioned.

The structures of the complexes are given in Fig. 32-35, with the Cl and 1C conformation given for the BD complex. Because it is difficult to convey by a drawing the proper relationships in the BD_2 complex, a photograph of a molecular model of the BD_2 complex of the 4-O-methyl-α-D-mannoside is presented in Fig. 36.

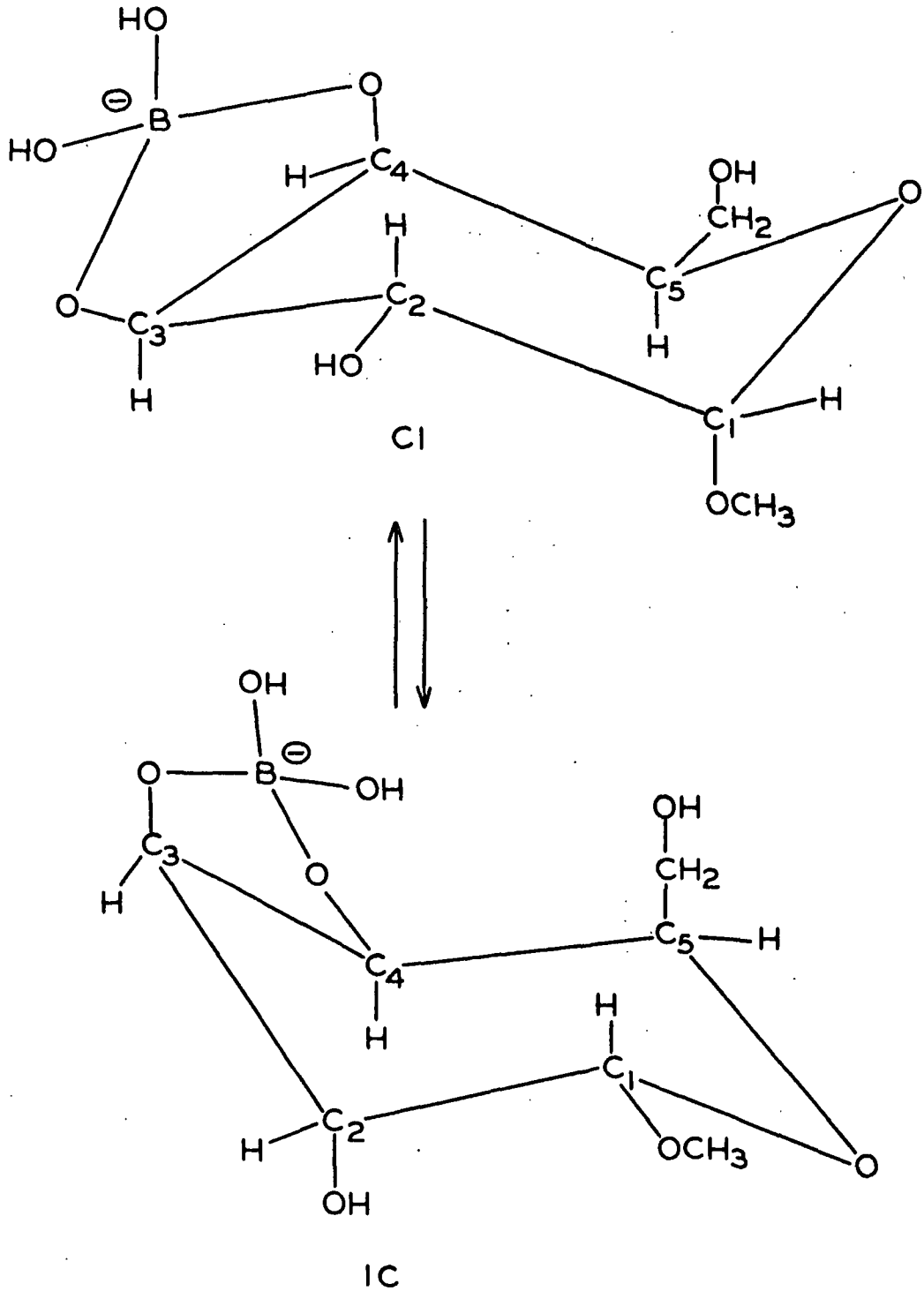


Figure 32. Proposed Structures of the Monocomplex of Methyl-α-D-galactopyranoside

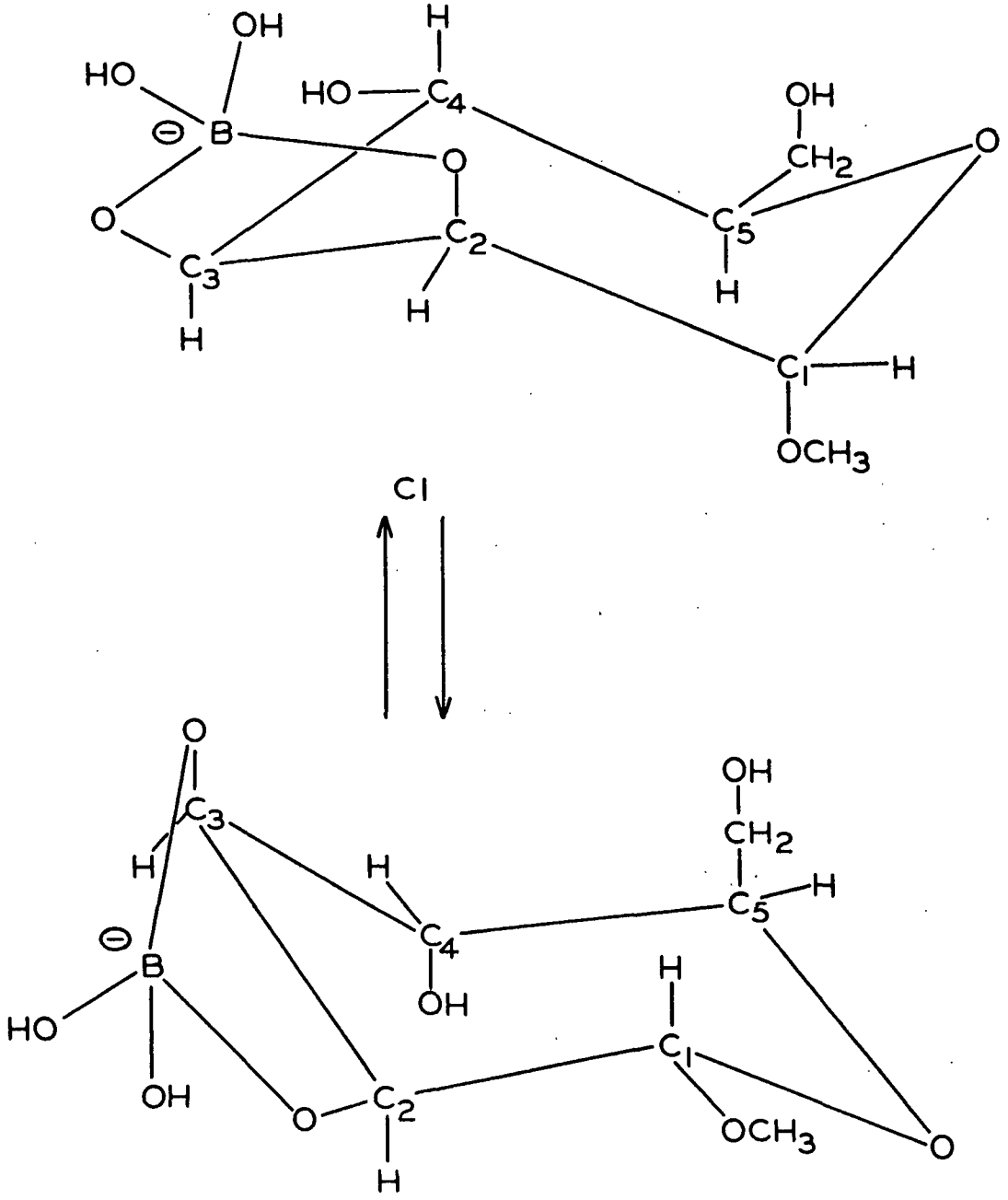


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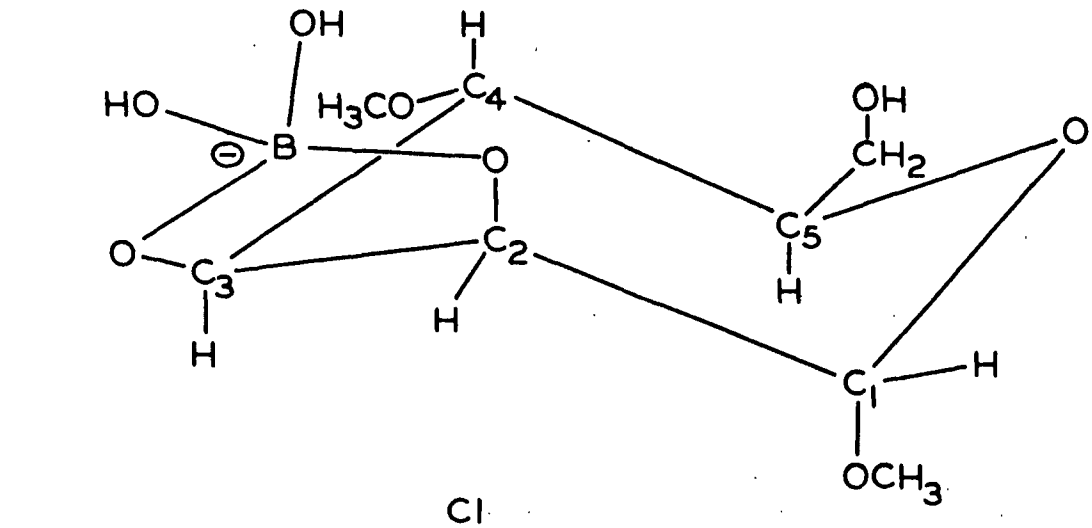
Figure 33. Proposed Structures of the Monocomplex of Methyl-α-D-mannopyranoside



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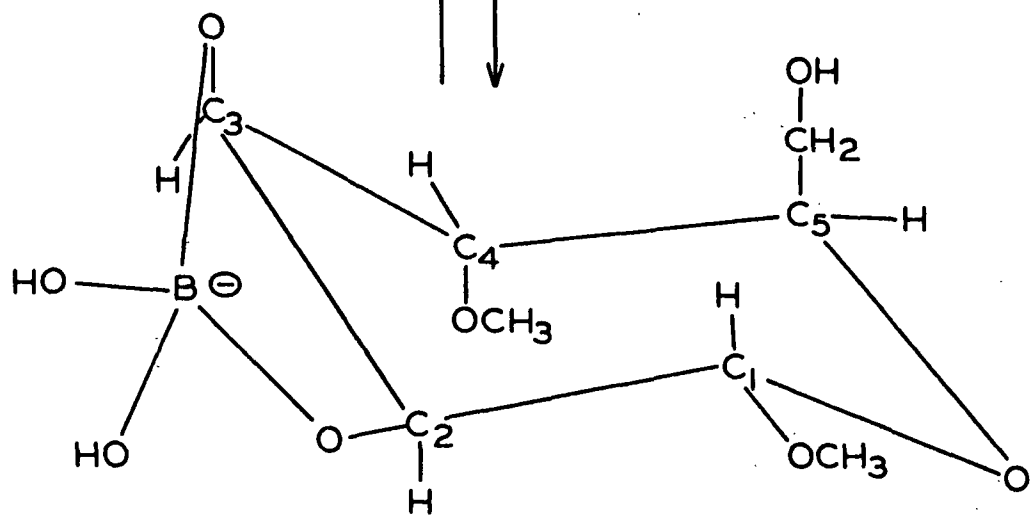

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Figure 34. Proposed Structures of the Monocomplex of Methyl-4-O-methyl-α-D-mannopyranoside

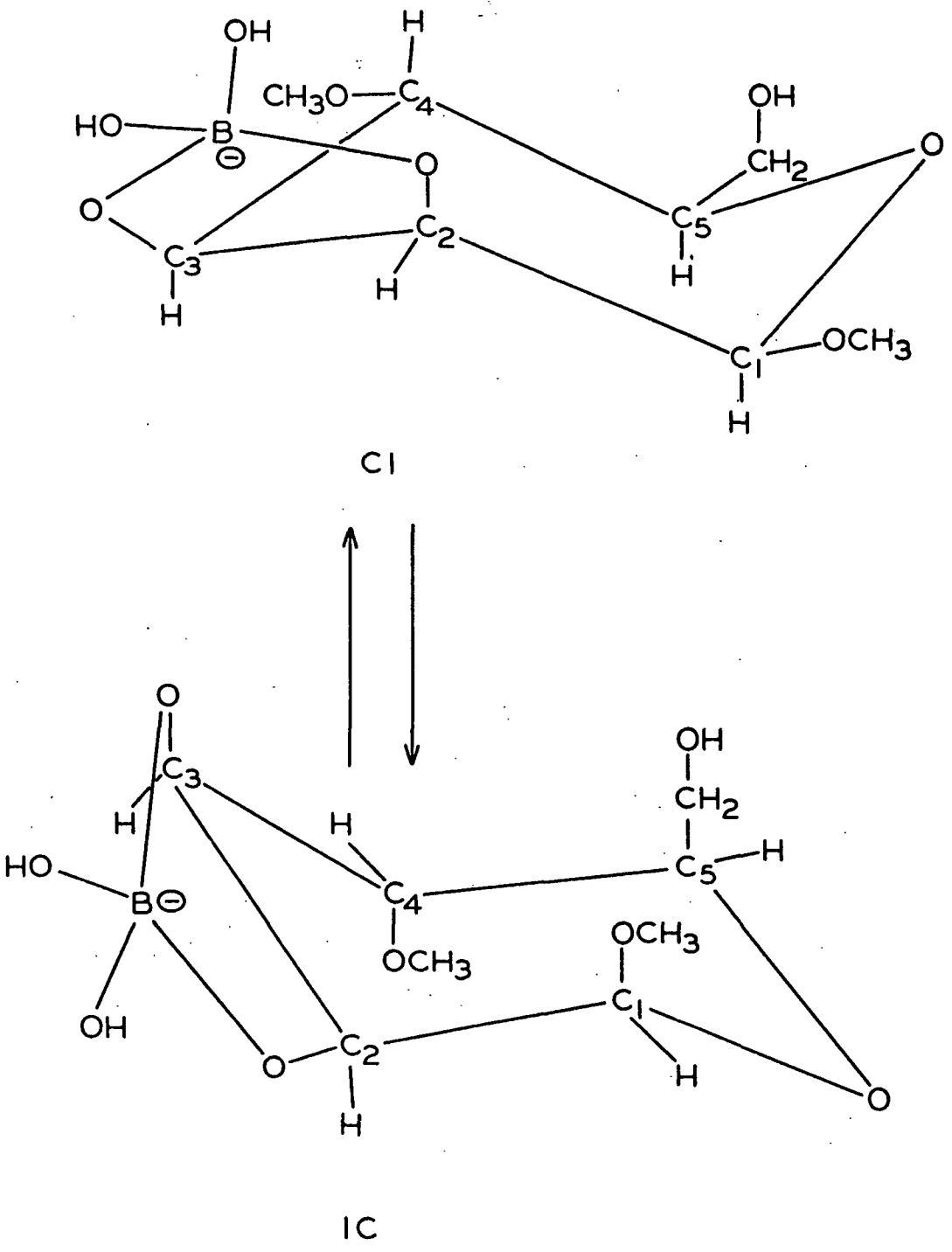
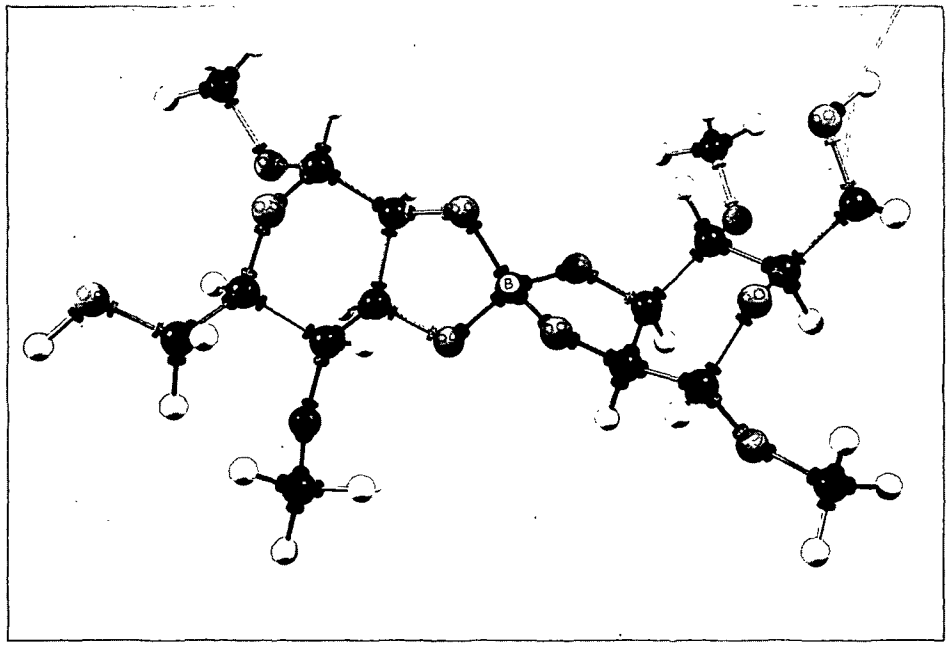
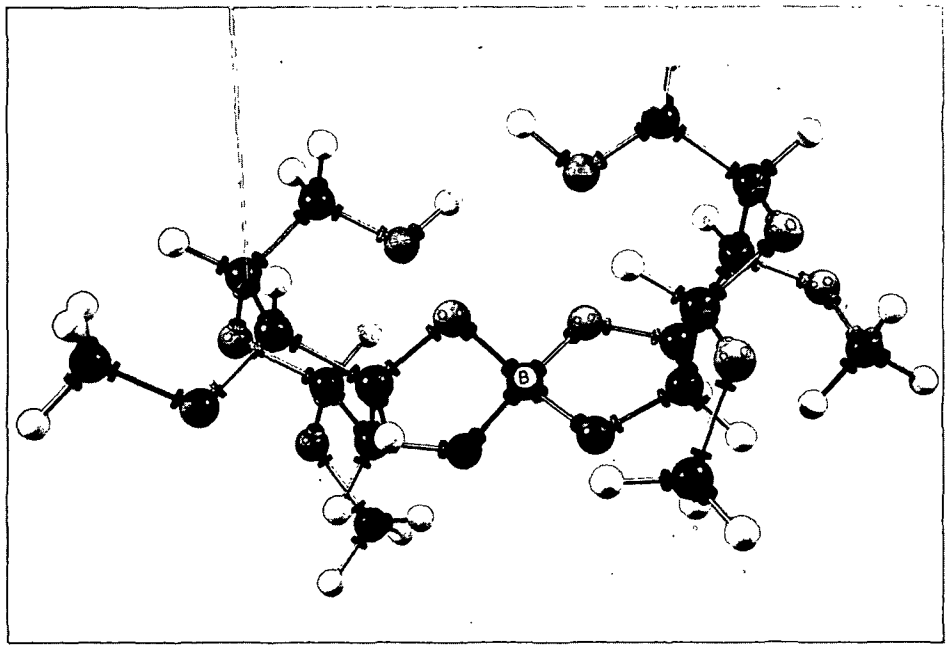


Figure 35. Proposed Structures of the Monocomplex of Methyl-4-O-methyl-β-D-mannopyranoside



C1



1C

white -- hydrogen
gray -- oxygen
black -- carbon

Figure 36. BD_2 Complex with Methyl-4-O-methyl-α-D-mannopyranoside

It illustrates the many interactions that are possible in the BD_2 complex. In the following discussion no attempt will be made to pinpoint exact interactions, but rather the general nature of the over-all effect will be presented.

TABLE XII

STABILITY CONSTANTS FOR THE MODEL COMPOUND—BORATE SYSTEM

Compound	β_1	β_2	K_2	K_T
Methyl-α-D-galactopyranoside	100	400	4	4×10^4
D-galactose[a]	120	300	2.5	3.6×10^4
Methyl-α-D-mannopyranoside	50	600	10	3×10^4
D-mannose[a]	50	490	10	2.5×10^4
Methyl-4-O-methyl-α-D-mannopyranoside	30	3000	100	1×10^5
Methyl-4-O-methyl-β-D-mannopyranoside	2	400	200	8×10^2

[a]Values obtained by Lorand and Edwards (28), using the pH method.

EFFECT OF STERIC FACTORS ON COMPLEX FORMATION

From a consideration of the stability constants, the α-galactoside and α-mannoside form the total borate complex with almost equal ability, while the α-mannoside forms the BD_2 complex more readily than the α-galactoside. This can be explained by considering that the α-galactoside complex forms across the C_3-C_4 hydroxyls where the C_5-CH_2OH group can easily interfere with the formation of the dicomplex, while with the α-mannoside, the complex forms across C_2-C_3 hydroxyls, where the complex can form without interference from the -CH_2OH group. In both cases the glycosidic -OCH_3 group is in an axial position in the preferred Cl chair conformation and is relatively remote from the site of complex formation.

The substitution of the C_4-OH on the α-mannoside to give the 4-O-methyl-α-mannoside favored complex formation. Not only was the highest value for K_T obtained with the 4-O-methyl-α-mannoside, but the stability of the dicomplex was also greatly increased. This is reasonable, as the addition of the C_4-OCH_3 group stabilizes the Cl chair conformation, with the C_4-OCH_3 group equatorial in this conformation. From a consideration of molecular models, the Cl chair form gives the complex with little interference from adjacent groups, while the 1C chair form brings both the C_1-OCH_3 and the C_4-OCH_3 groups into interfering positions. Therefore, groups which stabilize the Cl chair conformation should stabilize the complex, assuming no adverse effects occur for other reasons. The increase in the amount of complexing which occurs with the 4-O-methyl-α-mannoside also shows that the C_2-C_3 cis-hydroxyl groups are the site of complex formation in the mannose compounds, for if complexing across other adjacent hydroxyls was important, the 4-O-methyl-α-mannoside would have complexed less than the α-mannoside.

Foster (35) from ionophoresis work, proposed that the β-glycoside of mannose would not form a borate complex as readily as the α-glycoside due to the interference of the glycosidic -OCH_3 group. The present results support this supposition, as the total amount of complex dropped considerably from the α- to the β-glycoside of 4-O-methyl-D-mannose.

From a consideration of molecular models, the C_1-OCH_3 group does interfere with complex formation in the β-glycoside. Also, the unstabilizing effect of the C_2 axial oxygen may be increased due to complex formation across the C_2-C_3 hydroxyls. This effect will also be present in the 4-O-methyl-α-mannoside; but in 4-O-methyl-β-mannoside, the C_2-O valence bisects the tetrahedral angle of the two C_1-O valences, giving an enhanced unstabilizing effect (51).

Steric considerations do not explain why the 4-O-methyl-β-mannoside formed the dicomplex in marked preference to the monocomplex (see K_2 value). With the 4-O-methyl-β-mannoside, the CH_3O- and the C_6-OH groups are equatorial in the C1 chair conformation and hence favor the formation of the BD_2 complex. However, the C_1-OCH_3 group is in position to interfere with the formation of both the BD and BD_2 complexes and should diminish formation of both types of complex. It could be hypothesized that repulsive forces between sugar units lower the interference of the C_1-OCH_3 group in the monocomplex, but, as will be seen, differences in hydrogen bonding offer a more likely explanation for the small amount of BD present.

EFFECT OF HYDROGEN BONDING ON COMPLEX FORMATION

Intramolecular hydrogen bonds form between a hydrogen atom and an atom containing an unshared electron pair, with the strength of the bond best correlated with the acidity of the hydrogen atom and the basicity of the atom with the unshared electron pair (65). In the case of the borate complex, the boron atom carries a negative charge, and the oxygen atoms bonded to the boron can be expected to have increased electronegative character. Therefore, the hydrogen bonds form between the hydroxyl hydrogens of the sugar molecule and the oxygen atom bonded to the boron atom. These hydrogen bonds will tend to decentralize the negative charge and increase the stability of the complex. This stabilization is especially important in the monocomplex where the boron hydroxyl groups are relatively free to move and can readily come into position for hydrogen bonding.

The α-galactoside which has hydroxyl groups available on either side of the position of complex formation has the best opportunity of the model compounds

to form hydrogen bonds. The α-mannoside has hydrogen bonding opportunities with only one neighboring hydroxyl group and also across the ring to the C_6-OH group. The 4-O-methyl-α-mannoside has no adjacent hydroxyl groups and can only form hydrogen bonds across the ring with the C_6-OH group. In the case of the β-glycoside of 4-O-methyl-D-mannose, hydrogen bonding will be even less as the glycosidic methyl group interferes with bonding across the ring. Hydrogen bonding stabilizes the BD complex, hence the α-galactoside should form the most stable BD complex, with the α-mannoside, the 4-O-methyl-α-mannoside, and the 4-O-methyl-β-mannoside following in order. As can be seen from the values for β_1 (Table XII), this was the order obtained experimentally, supporting the hydrogen bonding hypothesis.

The relatively small β_1 value for the 4-O-methyl-β-mannoside can then be explained as due to the relative lack of hydrogen bonding to stabilize the BD complex. It appears that the C_6-OH group has an important stabilizing effect as a large change in β_1 did not occur until hydrogen bonding across the ring was hindered by the glycosidic methyl group of the β-glycoside. A consideration of molecular models also indicates that the C_6-OH group is the most likely point for hydrogen bonding. Steric hindrance of the actual complex formation also enters in, however, and as the relative magnitude of the two factors is not known, it cannot be said for certain whether the C_6-OH is the dominant point of hydrogen bonding.

Taken together, the postulated effects of hydrogen bonding and steric hindrance can reasonably account for the observed difference in stability constants. It can be seen in Fig. 37 that the relative order of hydrogen bonding and steric hindrance follows the order of the appropriate stability constant. Although the

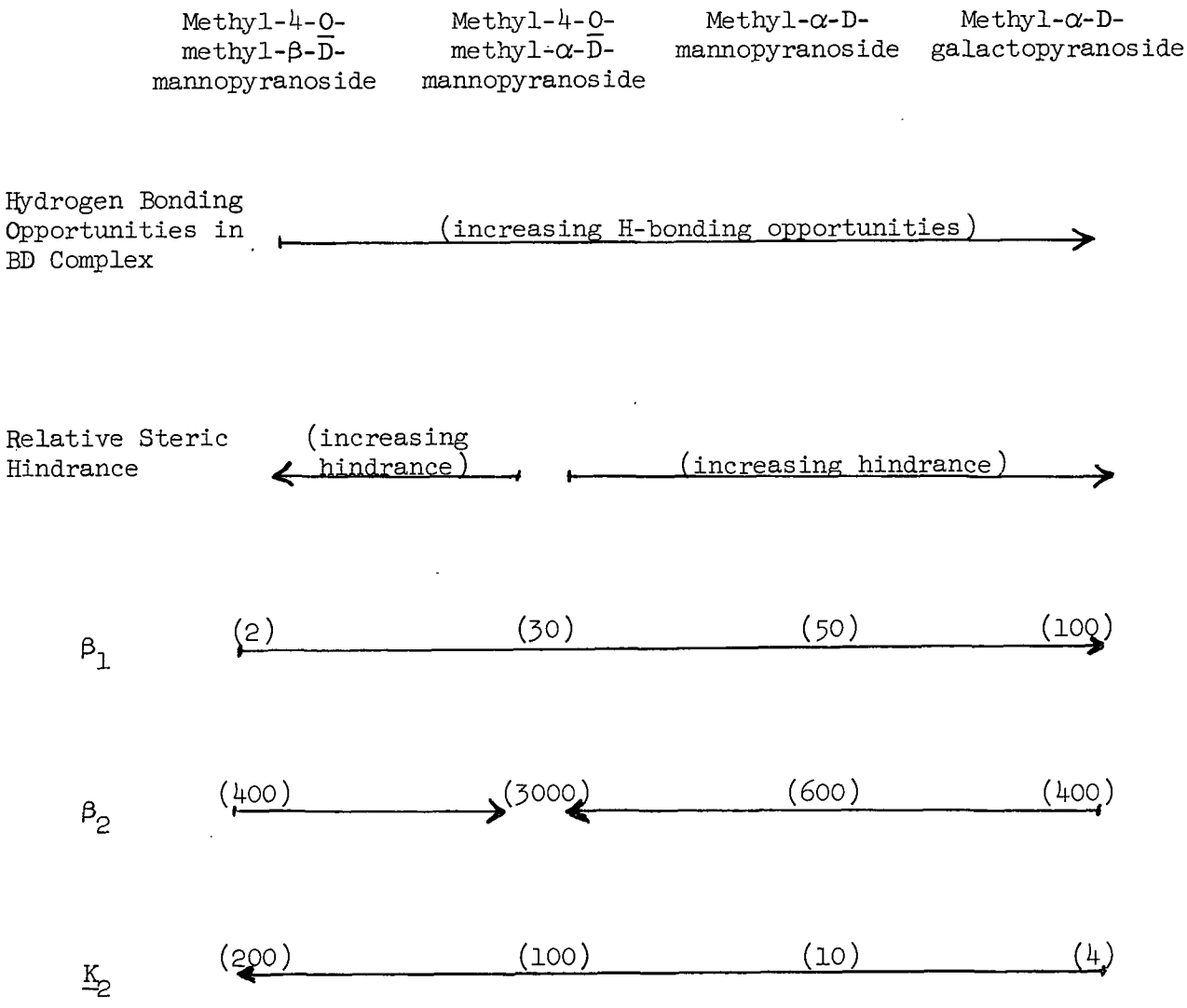


Figure 37. Relation of Stability Constants to Hydrogen Bonding and Steric Hindrance

absolute magnitude of each factor remains unknown, hydrogen bonding and steric hindrance serve to explain the relative relations between the stability constants.

COMPARISON WITH PREVIOUS RESULTS

The stability constants for D-galactose and D-mannose given in Table XII were determined by Roy and co-workers (28), using pH measurements. The close agreement of these values with the appropriate glycoside was somewhat unexpected as the pH work with the free sugars was not only complicated by the various equilibrium forms of the free sugar, but also by the presence of polyborate anions. The complications due to the different forms of the free sugar can be resolved by assuming that the complex forms with the relatively rigid ring form and is similar to that formed with the fixed glycosidic ring. However, if polyborate anions were a major factor, greater differences in the stability constants should have been noted. It therefore appears that polyborate anions are not as important a factor as was believed.

Of course, the possibility exists that any effect of the polyborate anion was cancelled by differences in structure between the free sugar and the glycosides, with the closeness of the stability constants due to pure chance. However, it seems likely that complex formation between polyborate anions and the carbohydrate would have a rather large effect on the stability constants, since a relatively large amount of boron atoms could be bound up in a single complex. This should cause a rather large error in the calculated value of the free borate concentration, greatly affecting the calculated stability constants for a system.

RELATION TO THE GALACTOMANNAN POLYMER

While several additional model compounds were included in the final study, the α-galactoside and the 4-O-methyl-β-mannoside are the model compounds

representing the galactomannan polymer. From these compounds, it appears that the borate complex forms more readily on the galactose side units than on the mannose backbone (see $\underline{K_T}$ values). However, the cross-linking site appears to be of equal likelihood on either the mannose or galactose unit (see β_2 values). The ability of the galactose unit to form the monocomplex will aid the solubility of the polymer in water. This offers an explanation for the poor solubility of straight-chain mannans in borate solutions in comparison to galactomannans. In general, the results obtained with the model compounds substantiate the view that the behavior of the galactomannan–borate system can be explained on a basis of two types of complexes being formed, that is, the dicomplex, which gives crosslinking, and the monocomplex, which gives increased aqueous solubility. Changes in experimental conditions, such as changes in the ratio of carbohydrate to borate, greatly effect not only the amount of complexing, but more important, the type of complex present. By selection of suitable conditions, the borate system can be made to give widely different results, i.e., gelation or solvation.

LIST OF MAIN SYMBOLS

A	general ligand of complex
$\underline{a}$	intensive factor for polyhydroxyl compound, D
$\underline{B}$	general central group of complex
B	borate anion, $B(OH)_4^-$
$\underline{B}_o$	initial amount of B
BD	monocomplex formed from one molecule of B and one molecule of D
BD_2	dicomplex formed from one molecule of B and two molecules of D
B_2D	diborate complex formed from two molecules of B and one molecule of D
$\underline{b}$	intensive factor for borate anion, B
$\underline{c}$	intensive factor for monocomplex, BD
D	polyhydroxyl compound containing an adjacent cis-hydroxyl group
$\underline{D}_o$	initial amount of D
$\underline{d}$	intensive factor for dicomplex, BD_2
E	borate ester
$\underline{e}$	intensive factor for potassium hydroxide
$\underline{K}_n$	stoichiometric step stability constant for the formation of BA_n from BA_{n-1} and A
$\underline{K}_T$	stoichiometric total stability constant for the formation of BA_n, BA_{n-1}, ... from B and A
KOH_o	initial amount of potassium hydroxide
$\underline{k}$	geometric constant of equipment
$\underline{M}$	sum of $[\underline{D}_o]$ and $[\underline{B}_o]$
$\underline{R}$	refractometer scale reading
$\Delta\underline{R}$	change in R due to complex formation
$\underline{X}_n$, $\underline{X}_a$	intensive factor of BA_n or A
$\underline{Y}$	general physical property of a solution

$\underline{Y}_o$	$\underline{Y}$ value of pure solvent
$\underline{Y}_{\underline{nc}}$	$\underline{Y}$ value if no complex forms
$\Delta\underline{Y}$	change in $\underline{Y}$ due to complex formation
α	observed optical rotation
$\Delta\alpha$	change in α due to complex formation
$\beta_{\underline{n}}$	stoichiometric over-all stability constant for the formation of $\underline{BA}_{\underline{n}}$ from $\underline{B}$ and $\underline{A}$
[ ]	concentration in mole/liter

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APPENDIX I

PREPARATION OF MODEL COMPOUNDS

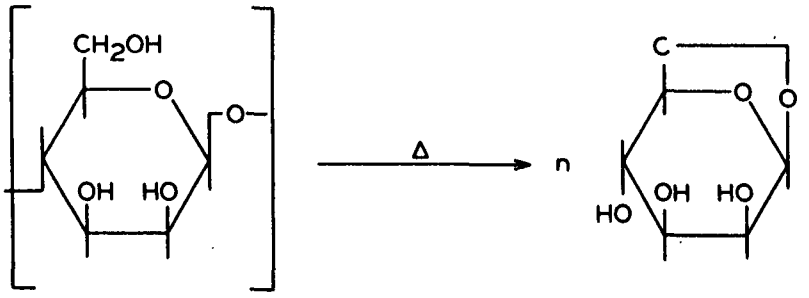
METHYL-α-D-GALACTOPYRANOSIDE

D-galactose was converted to the galactoside by refluxing in methanol for 14 hours, using 1% HCl as a catalyst. Three batches of 50 g. galactose each were processed. After the reflux period, the acid was neutralized with silver carbonate, and the resulting precipitate was removed by filtration. A small amount of decolorizing carbon was also added and removed by filtration. The clear methanol was then concentrated under vacuum to a thick sirup. Upon dissolving the sirup in absolute isopropanol, the α-galactoside should have crystallized from this solution. However, in two tries at this procedure, only a gum-like precipitate could be obtained. Since Dale and Hudson (47) recrystallized their isopropanol product from absolute ethanol, the isopropanol solution was concentrated to a thick sirup and redissolved in absolute ethanol. A crystalline product was then obtained. The β-galactoside crystallized first, with the α-galactoside obtained from the mother liquor. On recrystallization from ethanol, the pure α-galactoside was obtained.

MANNOSE DERIVATIVES

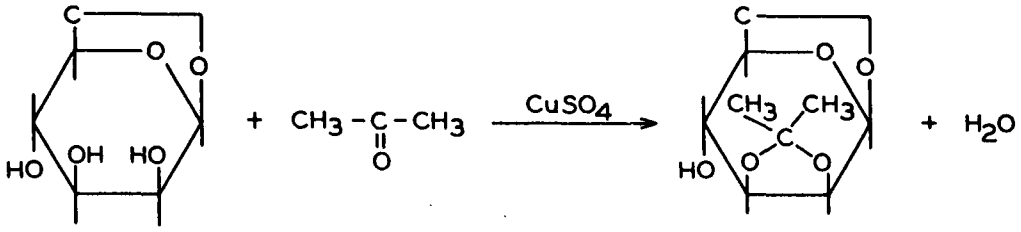
2,3-ISOPROPYLIDENE-D-MANNOSAN

The acetone derivative of mannosan was prepared, following the procedures of Knauf, Hann, and Hudson (66). The following equations illustrate the main features of the procedure.



(86)

D-MANNAN D-MANNOSAN <1, 5>β <1, 6>



(87)

D-MANNOSAN <1, 5>β <1, 6> 2, 3-ISOPROPYLIDENE-D-MANNOSAN <1, 5>β <1, 6>

Ivory nut meal, prepared from the original ivory nuts of the tagua palm, was used as a source of mannan. This meal was prepared by the following procedure.

1. The rough ivory nuts were soaked in a 2N NaOH solution for 4 hours at room temperature.

2. The loose outer skin was removed, and the inner skin was scraped off.

3. After washing to remove the remaining traces of caustic, the cleaned nuts were reduced to fine shavings by turning down on a lathe.

4. The shavings were soaked overnight in distilled water and given a final washing with acetone before being dried in a vacuum desiccator.

An alternate method of reducing the nuts to a meal was used on several cracked nuts and pieces from the lathe operation. These pieces were cracked with a hammer into pieces about 1/4 inch in diameter and fed through a small Wiley mill,

using a 20-mesh screen on the mill. The sandlike meal was processed in the same manner as the lathe shavings, with no difference noted between the two meals.

Pyrolysis, under reduced pressure, was used to obtain the D-mannosan $\langle 1,5 \rangle$ β $\langle 1,6 \rangle$ from the ivory nut meal. The mannosan itself was never isolated, but was used directly in the production of the acetone derivative. A diagram of the apparatus used in this procedure is given in Fig. 38.

Six separate batches of ivory nut meal were used, each batch containing an equivalent of approximately 100 g. of ovendry meal. A total amount of 602.5 g. of ovendry meal were used. Pyrolysis was carried out using the luminous flame of a Mekker burner, with the pressure in the system held close to 30 mm. of mercury. Between batches, the condensate was removed from the large flask, and the delivery system was cleaned.

The combined condensate was dissolved in approximately 900 ml. of distilled water and filtered through 20 g. of Celite and through 90 g. of decolorizing carbon. The latter filtration was of major importance as it removed impurities and allowed the preparation of a crystalline derivative.

The aqueous solution was concentrated under vacuum to a thin sirup and mixed with 500 ml. of acetone. After standing overnight, the clear acetone solution was decanted from a small amount of gumlike deposit. Fifty grams of anhydrous $CuSO_4$ were added to the acetone solution, and the mixture was shaken for 24 hours.

After removing the $CuSO_4$ by filtration, the acetone solution was concentrated under vacuum. At several stages in the concentration a precipitate formed in the solution. These precipitates were removed by filtration, with the

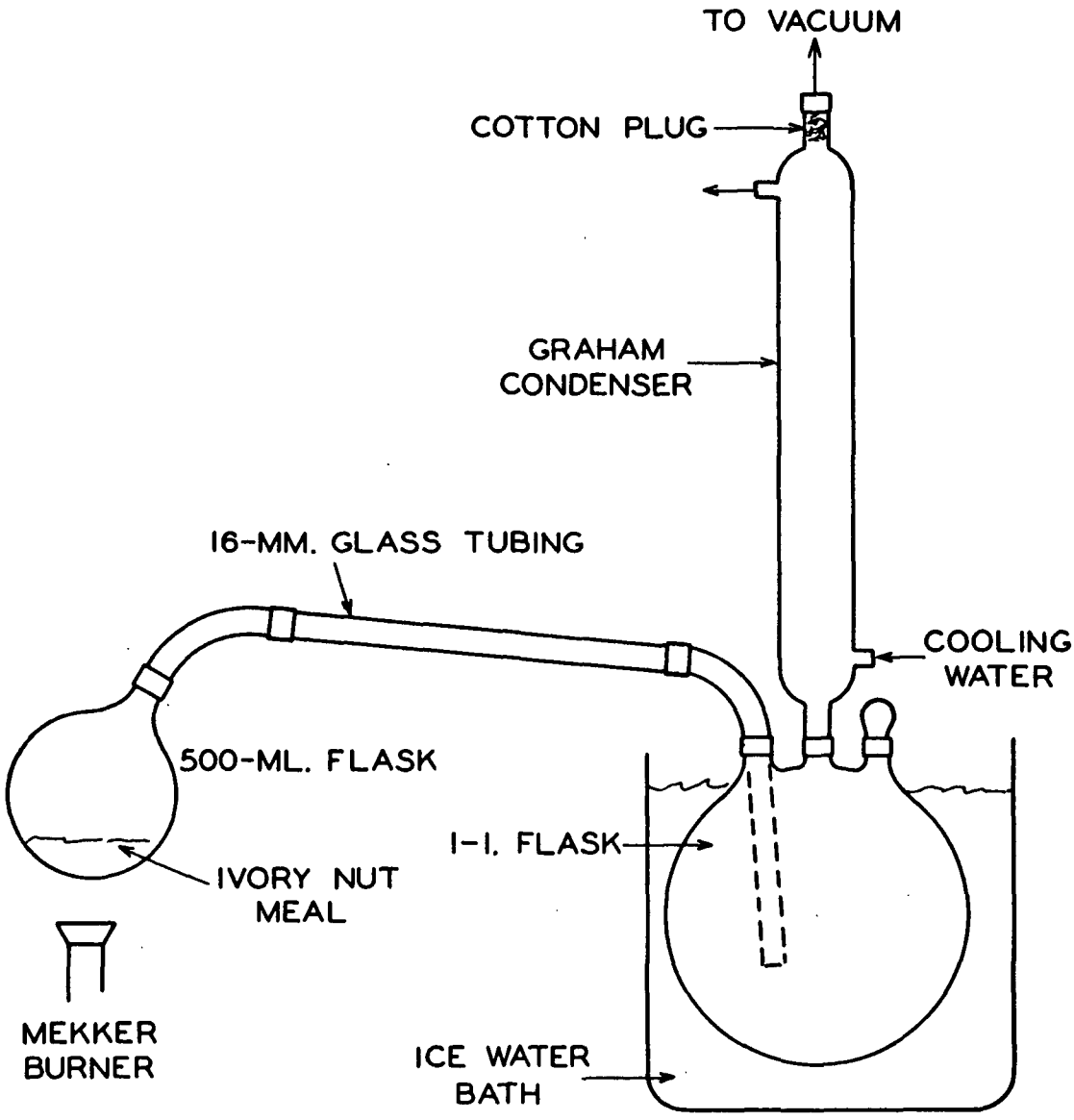


Figure 38. Apparatus for the Pyrolysis of Ivory Nut Mannan

concentration continued down to a small amount of thick sirup. The precipitates were washed with absolute isopropanol and recrystallized from n-butanol. A small amount of product was recovered from the isopropanol washings, and was also recrystallized from n-butanol. During recrystallization, long, needle-shaped crystals of 2,3-isopropylidene-D-mannosan ⟨1,5⟩ β ⟨1,6⟩ were obtained. The physical data are given below:

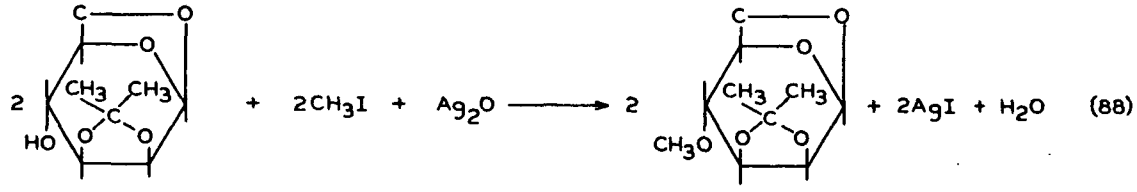
(1) Total yield, 26.14 g., or 4.33% of initial meal,

(2) Melting point, 160-161°C. (literature, 161-162°C.) (66),

(3) Specific rotation, -58.6° in H_2O at 589 mμ (literature, -58.8°) (66).

4-O-METHYL-2,3-ISOPROPYLIDENE-D-MANNOSAN ⟨1,5⟩ β ⟨1,6⟩

The 4-O-methyl compound was prepared by methylation of the isopropylidene-mannosan compound according to the procedure of Knauf, Hann, and Hudson (66). In this procedure, methyl iodide was used as the methylating agent, with silver oxide present as a catalyst. The reaction can be represented as follows:



2, 3-ISOPROPYLIDENE-D-MANNOSAN<1, 5>β<1, 6>

4-O-METHYL-2, 3-ISOPROPYLIDENE-D-MANNOSAN<1, 5>β<1, 6>

Twenty-five grams of the isopropylidene-mannosan were dissolved in a mixture of 25 ml. of acetone and 25 ml. of methyl iodide. Twenty-five grams of non-indicating drierite were added, and the mixture was set to reflux on a combination stirrer-hot plate. Four equal portions (11.25 g. each) of silver oxide were added down the reflux condenser at 15-min. intervals. At the end of the silver

oxide addition, the condenser and the neck of the reaction flask were washed down with 20 ml. of the acetone–methyl iodide mixture. The solution was then refluxed for 12 hours, with the reaction flask covered with aluminum foil.

At the end of the reflux period, the solids were removed by filtration, the precipitate was washed with three small quantities of acetone, and the combined filtrate and washings were concentrated under vacuum to a thick sirup. This sirup was dissolved in 20 ml. of ether, mixed with 120 ml. of n-pentane, and placed in the freezer overnight.

No precipitate was obtained in the ether–pentane solution. Therefore, the solution was reconcentrated to a sirup. This sirup was redissolved in 20 ml. of ether, and after the addition of 100 ml. of n-heptane was placed in the freezer.

A small amount of the ether–pentane solution was saved and allowed to evaporate from the walls of a 500-ml. flask. Crystal sheets were obtained on the sides of the flask and were used to seed the ether–heptane solution.

After 8 hours, a large mass of crystals grew in the ether–heptane solution. The crystals were separated by filtration, with the mother liquor concentrated and reworked from an ether–heptane solution. This reworking of the mother liquor was repeated until negligible solids remained when the final mother liquor was evaporated to dryness.

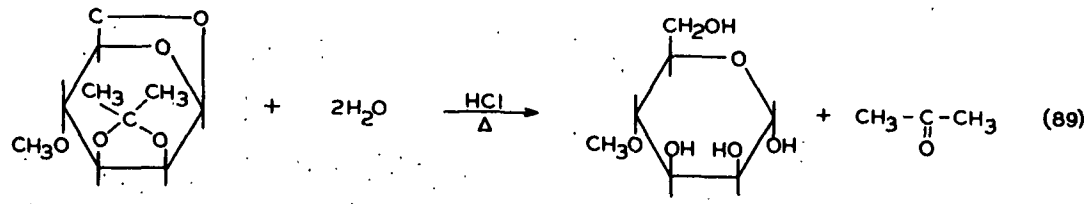
Total crude yield of 4-O-methyl-2,3-isopropylidene-D-mannosan $\langle 1,5 \rangle$ β $\langle 1,6 \rangle$ was 24.27 g. air dry (89.1% of theoretical). The crude product was recrystallized from ether–heptane to give the pure material. The physical properties were as follows:

(1) Melting point, 52.5-54.0°C. (literature, 53-54°C.) (66),

(2) Specific rotation, -34.8° in $CHCl_3$ at 589 mμ (literature, -33.4°) (66).

4-O-METHYL-D-MANNOSE

The acid hydrolysis procedure of Haskins, Hann, and Hudson (49) was used to prepare 4-O-methyl-D-mannose from the methylated isopropylidene compound. The reaction can be represented as follows:



4-O-METHYL-2, 3-ISOPROPYLIDENE-D-MANNOSAN <1, 5>β <1, 6>

4-O-METHYL-D-MANNOSE

The hydrolysis reaction was carried out in 1N hydrochloric acid at 95°C. for 5 hours with constant stirring. Initially, the methylisopropylidene-D-mannosan (22.29 g.) did not dissolve in the acid solution (225 ml.), but, after 10 minutes of heating, all the material had gone into solution. After 5 hours, the solution was neutralized with silver carbonate, followed by filtration to remove the silver chloride precipitate. The filtrate was then concentrated in vacuum to a dry sirup, dissolved in 44 cc. of warm dioxane, and placed in a water bath at 13°C. for crystallization.

It should be noted that some trouble was encountered in removing the silver chloride from solution. This necessitated several passes through carbon-celite filters, with some loss in product.

Only a few small crystals were obtained from the dioxane solution. Therefore, the solvent was changed to absolute ethanol. The ethanol solution was placed in the refrigerator to see if crystals would form. Crystallization did occur, but at a very slow rate. In a 4-month period, 9.65 g. of recrystallized

4-O-methyl-D-mannose were recovered. This amounted to a 44.7% yield, compared to the expected 78% yield (49).

Paper chromatographic analysis of the ethanol mother liquor showed that a considerable amount of 4-O-methyl-D-mannose was still present, with mannose as the main impurity together with small traces of pentoses and higher methylated products. The remaining 4-O-methyl-D-mannose was separated by column chromatography and crystallized from ethanol. The chromatography procedure is discussed in a later section of Appendix I.

GLYCOSIDES OF D-MANNOSE BY DIRECT ALKYLATION

The procedure of Isbell and Frush (52) was followed, except for changes in the amounts of reactants and the alkalinity of the solution. An aqueous solution of mannose at the desired concentration was cooled to 0°C. and held at this temperature unless otherwise desired. Dimethyl sulfate was added dropwise to the rapidly stirred mannose solution. Depending on the amount added, the addition time varied from 1 to 3 hours, with the solution held alkaline by dropwise addition of 10N sodium hydroxide. As the reaction continued, the caustic was added at a rate necessary to control the pH of the solution at the desired level. To ensure that the solution did not become acidic, the total amount of alkali added was always in slight excess of theoretical. The addition time of the caustic solution varied from 6 to 8 hours. After the reaction was complete, the alkaline solution was placed overnight in the refrigerator.

The alkaline solution was neutralized with 6N sulfuric acid, treated with decolorizing carbon, filtered, and concentrated in vacuum to 30 ml. During evaporation, a small amount of barium carbonate was added to prevent the solution from becoming acidic. The water bath temperature was held near 50°C. during

concentration. The thin sirup was mixed with 60 ml. of dioxane and reconcentrated to facilitate dehydration, with the resulting thick sirup dissolved in 60 ml. of pyridine. After the solution was cooled, the precipitated salts were removed by filtration. The mannoside was then acetylated by the slow addition of 60 ml. of acetic anhydride.

After standing overnight at room temperature, the acetylated mixture was poured into an ice-water mixture, with the resulting gummy precipitate extracted thrice with chloroform. The chloroform solution was washed with successive solutions of sodium bicarbonate (until acid free), copper sulfate (until pyridine free), distilled water, and then dried, using drierite.

Following decolorizing and filtration, the dry chloroform solution was concentrated to a thin sirup which was dissolved in 100 ml. of ethanol and reconcentrated. The resulting sirup was dissolved in 100 ml. of ether. Petroleum ether was then added to saturation, and the solution placed in the refrigerator.

If the ethanol sirup was sufficiently free of impurities, it crystallized spontaneously to a solid mass. In this case, the crystals were thoroughly triturated with ether and separated by filtration. The ether filtrate was then treated in the same manner as when no crystals were obtained. These ether-insoluble crystals proved to be almost pure methyl-tetra-acetyl-β-D-mannopyranoside.

After standing overnight in the refrigerator, the ether—petroleum ether solution yielded a mixture of α- and β-methyl-acetates. These crystals were filtered off and thoroughly stirred with 100 ml. of ether, dissolving the α-methyl-acetate. Upon filtration and recrystallization from 95% ethanol, the pure methyl-tetra-acetyl-β-D-mannopyranoside was obtained.

The acetylated glycoside was dissolved in cold methanol and catalytically deacetylated with barium methylate (67), which required that the solution stand at 0°C. for 24 hours. After decomposition of the barium methylate and filtration, the methanol solution was concentrated to a sirup, dissolved in warm isopropanol, and placed in the refrigerator. After standing overnight, crystals of methyl-β-D-mannopyranoside isopropyl alcoholate were obtained, which were recrystallized from hot isopropanol and dried over calcium chloride. On heating in a vacuum oven at 105°C. the alcoholate decomposed, yielding a glass of pure methyl-β-D-mannopyranoside.

In cases where the recovery of the α-mannoside was desired, the ether solution of the ether-soluble acetate was first concentrated to a thick sirup and then deacetylated in the same manner as the β-methyl acetate. The methyl-α-D-mannopyranoside was obtained by crystallization from methanol.

All products, both crystalline and sirup, were analyzed by paper chromatography. A butanol:ethanol:water (5:1:4) (top layer) mixture was used as the developer, with the separation run on Whatman no. 1 paper. All samples were run in duplicate on separate sheets. One sheet was sprayed with aniline phthalate as a color reagent, while the second was sprayed with periodate-permanganate reagent. The former reagent showed the presence of reducing end groups, while the latter was a general reagent for hydroxyl pairs. In this manner, the glycosides were readily distinguishable from the nonglycosides. As long as the sheets were allowed to develop for approximately the same length of time (about 22 hours), the results were reasonably consistent.

PREPARATION OF THE CHROMATOGRAPHY COLUMN

Because good separation of the glycoside mixture had been obtained on Whatman no. 1 paper, the same substrate was used to prepare the column, using the following procedure.

Thoroughly washed Whatman no. 1 cellulose powder was dispersed in 50% ethanol. The cellulose column was formed by allowing the ethanol dispersion to filter slowly through a glass wool mat on the bottom of the glass column. When the liquid level had dropped below the top leg (see Fig. 39), the leg was removed, the air pressure (5 p.s.i.) was applied to aid in the compaction of the column. The column was washed with 95% ethanol until the initial 50% ethanol was removed.

The developer used in column chromatography was the top layer of a n-butanol: ethanol:water (5:1:4) mixture. The developer was prepared at 10°C. to ensure that no phase separation would occur on the column. The column was washed overnight with the butanol developer to displace the 95% ethanol. The refractive index of the effluent was checked to ensure that all the 95% ethanol had been removed. Dye rings were run to determine column uniformity. The final 2 by 20-inch cellulose column was suitable for separating from 3 to 5 grams of material (50).

The glycoside mixture was concentrated to a thick sirup, dissolved in n-butanol, and reconcentrated. The final sirup was dissolved in 20 ml. of the butanol developer, giving a clear solution. After dropping the liquid level in the column to the top of the cellulose, the glycoside solution was applied to the column. The layer of glycoside solution was taken into the cellulose by again dropping the liquid level to the top of the cellulose. Ten milliliters of developer were carefully applied, and the liquid level was again dropped; this was repeated three times. The reservoir of developer was then placed in position, and the run was started.

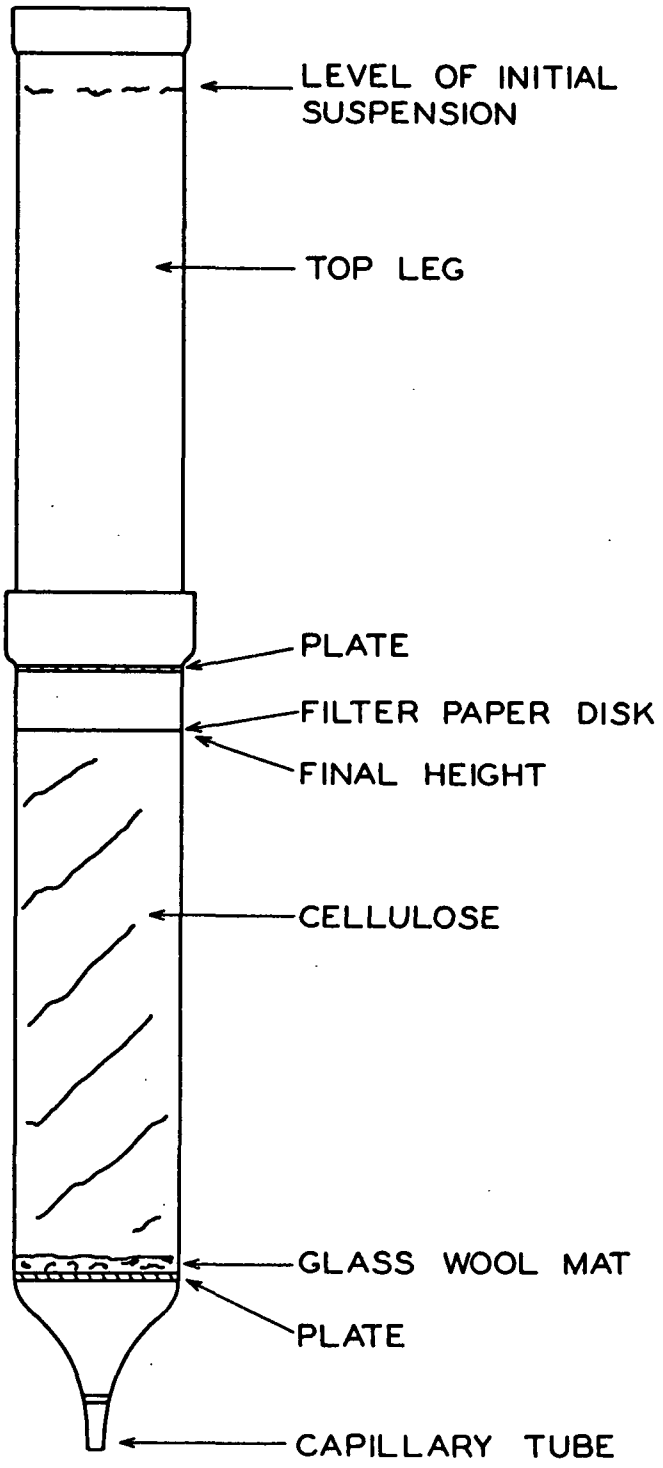
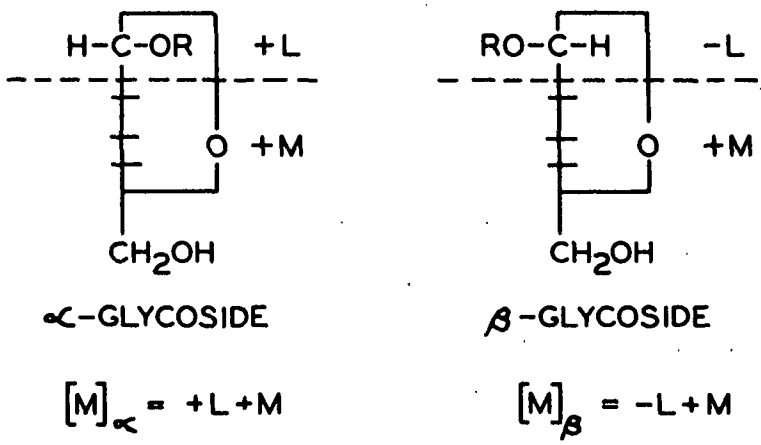


Figure 39. Cellulose Column Used in Separations

CALCULATION OF THE SPECIFIC ROTATION OF METHYL-4-O-METHYL-β-D-MANNOPYRANOSIDE BY HUDSON'S ISOROTATION RULES

The specific rotation of the 4-O-methyl-β-mannoside was estimated by the use of Hudson's isorotation rules (51). These rules are based on the assumption that the total optical rotation of a molecule is the sum of the rotations caused by each asymmetric center. Accordingly, the rotation of a glycoside is considered to be composed of two parts: L, the partial rotation of the anomeric carbon atom, and M, the rotatory contribution from the other active centers. Depending on the configuration, L and M can be positive or negative, with the net rotation of the glycoside equal to the sum of L and M. This is illustrated below.



Here [M] represents the molecular rotation of the glycoside, and it is easily shown that $[M]_\beta = [M]_\alpha - 2L$. Hudson stated that the rotation from the anomeric carbon is affected in only a minor degree by changes in the structure of the remainder of the molecule. Therefore, the value of 2L from the methyl glycosides of D-mannose can be used with the molecular rotation of the α-glycoside of 4-O-methyl-D-mannose to calculate the molecular rotation of the 4-O-methyl-β-mannoside. The calculations are given below.

$$2L = 28{,}930 \text{ (51)} \qquad (90)$$

$$[M]_{4\text{-}\underline{O}\text{-methyl-}\alpha\text{-mannoside}} = 17,460 \ (\underline{49}) \qquad (91)$$

$$[M]_{4\text{-}\underline{O}\text{-methyl-}\beta\text{-mannoside}} = 17,460 - 28,930 = -11,470 \qquad (92).$$

The specific optical rotation, $[\alpha]$, can then be calculated as follows:

$$[\alpha] = \frac{[M]_{\beta}}{\text{mol. wt.}} = \frac{-11,470}{208.2} = -55.1^{\circ} \qquad (93).$$

APPENDIX II

DETERMINATION OF REFRACTIVE INDEX AND OPTICAL ROTATION CONSTANTS AND VERIFICATION OF RESULTS

REFRACTIVE INDEX CONSTANTS FOR KOH AND $KB(OH)_4$

The KOH constant e and the borate constant b were determined as the initial step in this study. Equations (28) and (31) describe the necessary relations, with the experimental results given in Fig. 40 and 41. The IBM 1620 computer was used for these as well as all other slope calculations to give a least squares fit to the data points. Unless otherwise mentioned, the statistical F-test was used with a 95% confidence interval as a test for significance. The value of e was found to be 29.7, while the value of b was 44.7. These values for the refractive index constants were used throughout the study.

Because KOH and $KB(OH)_4$ have no effect on optical rotation, the values of b and e for the optical rotation measurements were zero. Hence, the optical rotation calculations were somewhat simpler than the refractive index equations.

METHYL-α-D-GALACTOPYRANOSIDE

DETERMINATION OF CONSTANTS

The D constant a, the BD constant c, and the BD_2 constant d were determined using the relations given in Equations (29), (35), and (39). The results of the refractive index and optical rotation measurements are given in Fig. 42-47. It should be noted that c was determined at a constant $[B_o]$ of 0.100 mole/liter, while d was determined at a constant $[D_o]$ value of 0.100 mole/liter. These same conditions were used in the c and d determinations with all model compounds.

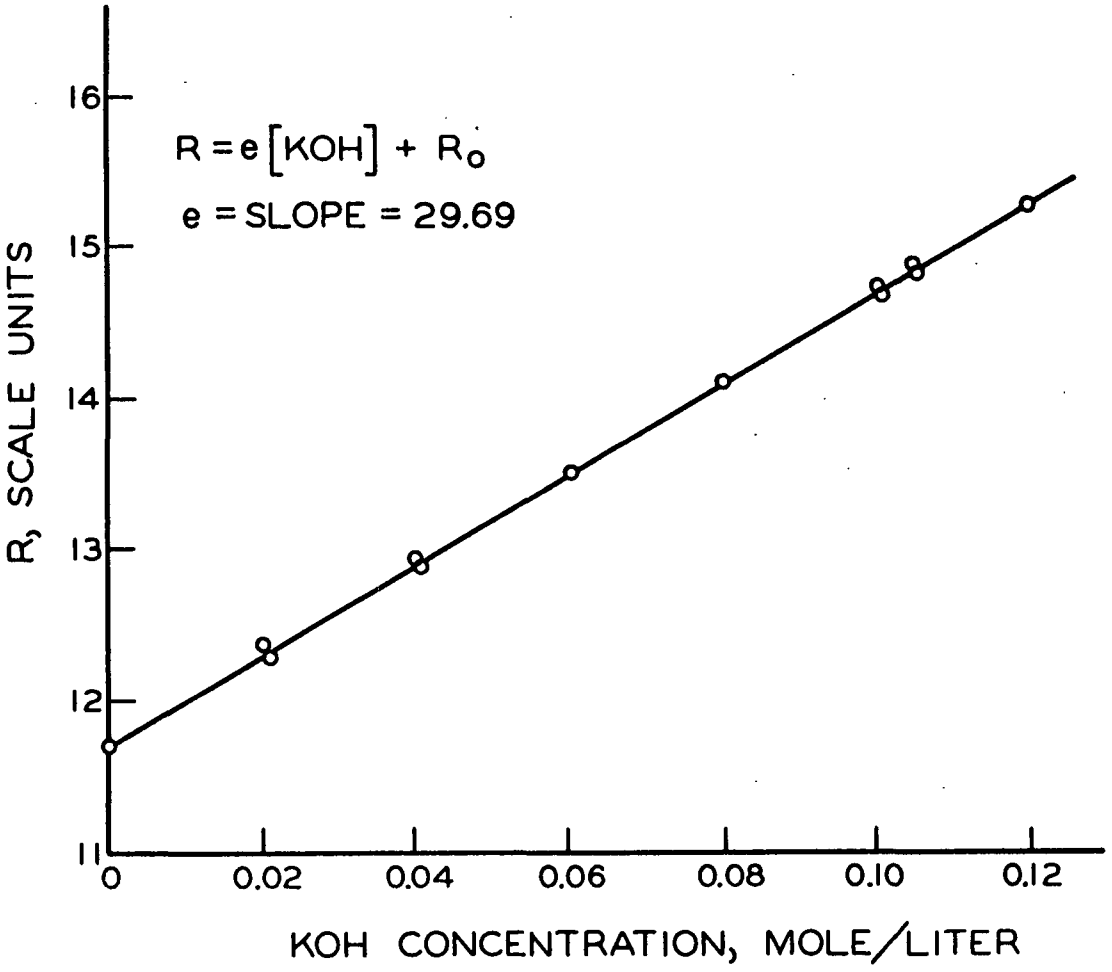


Figure 40. Determination of the KOH Constant e

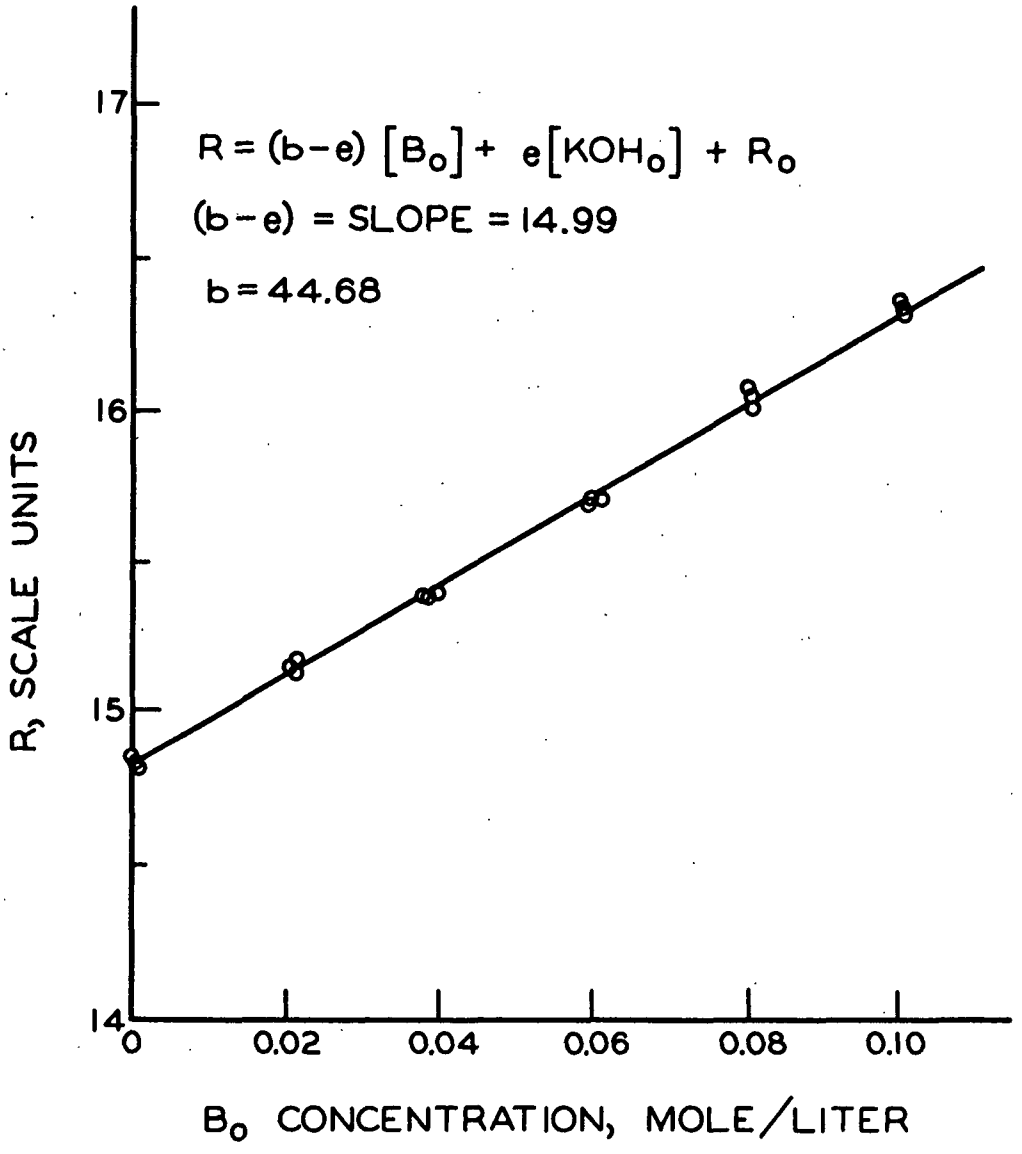


Figure 41. Determination of the Borate Constant b

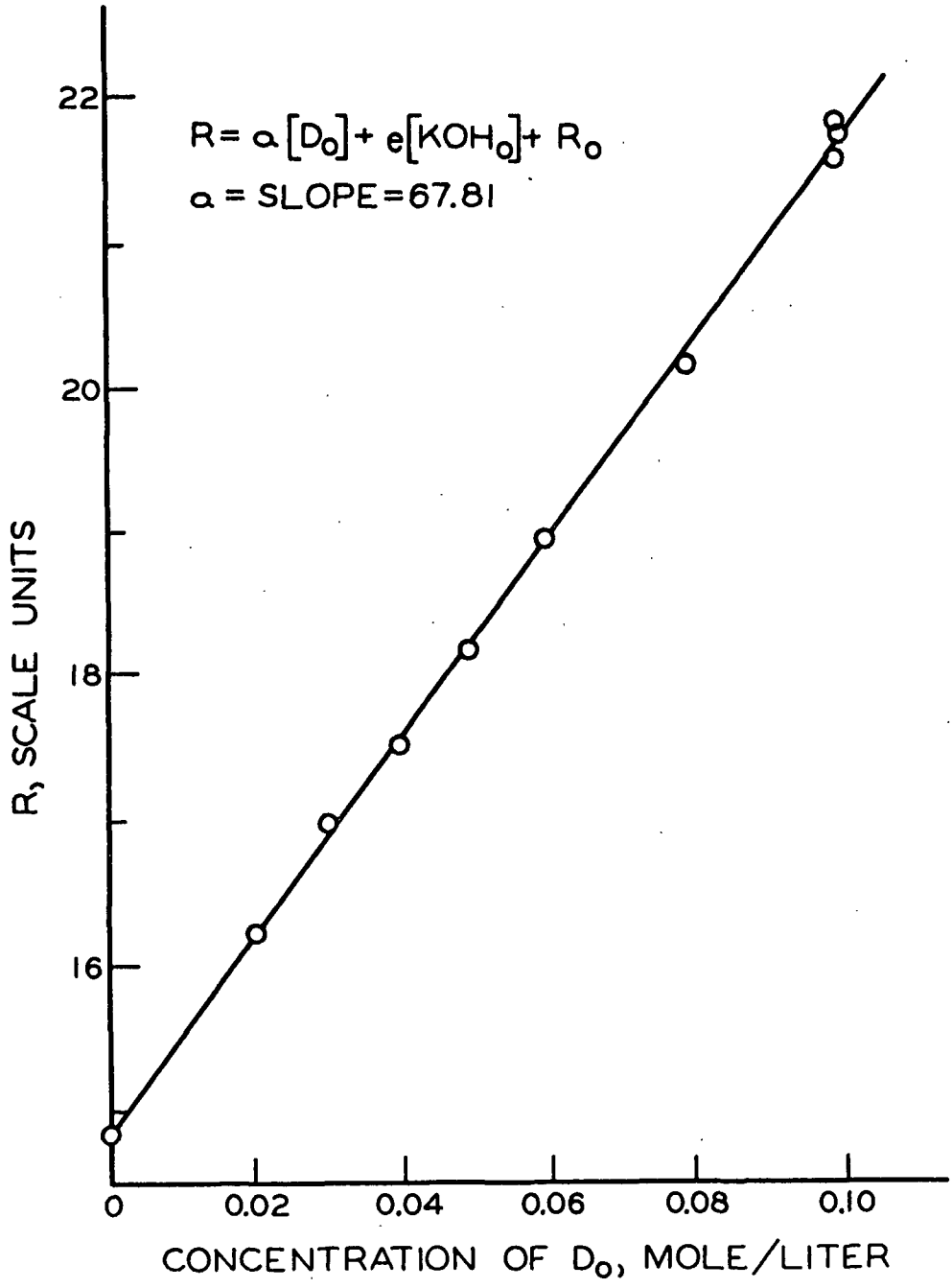


Figure 42. Determination of the Refractive Index Constant a for Methyl-α-D-galactopyranoside

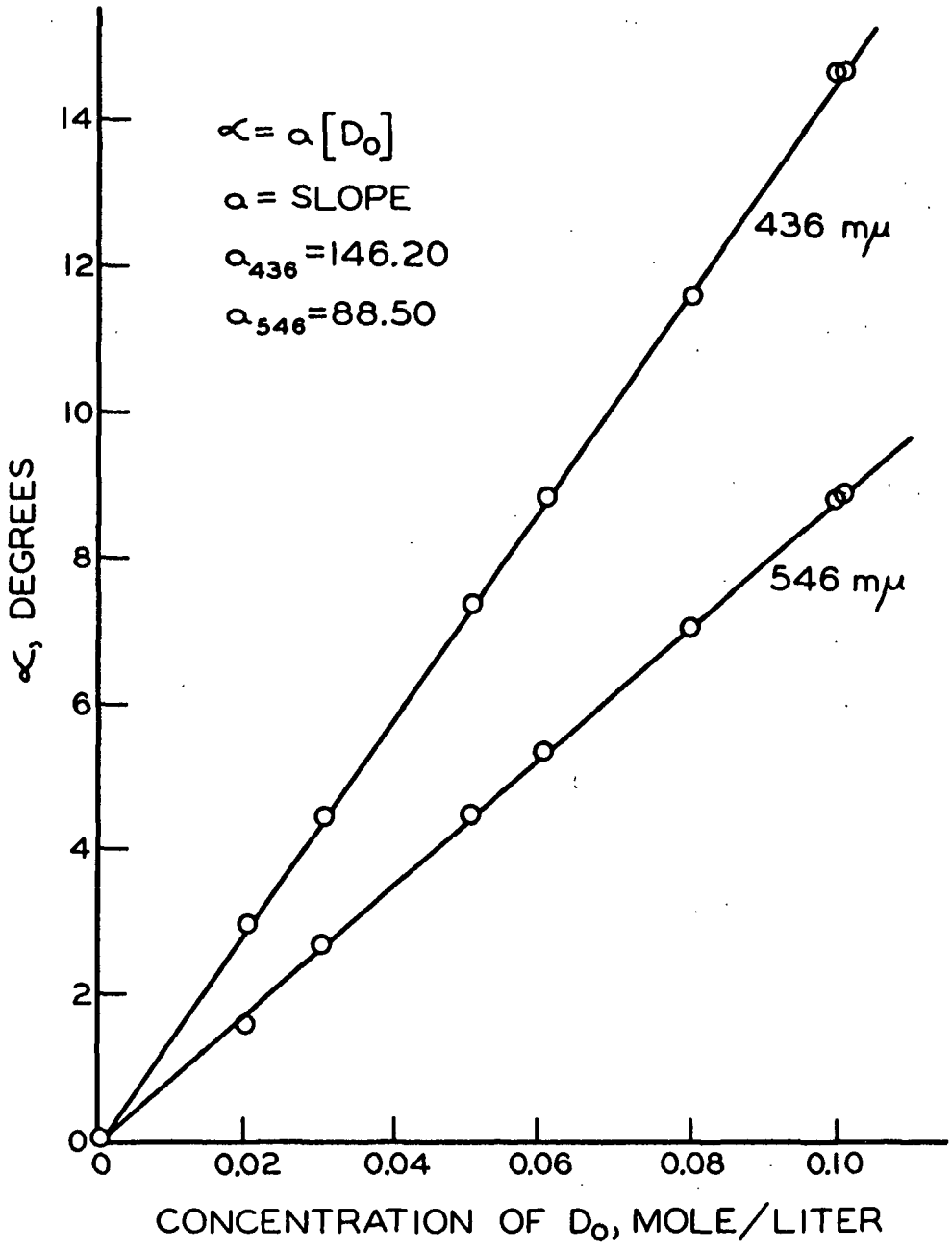


Figure 43. Determination of the Optical Rotation Constant a for Methyl-α-D-galactopyranoside

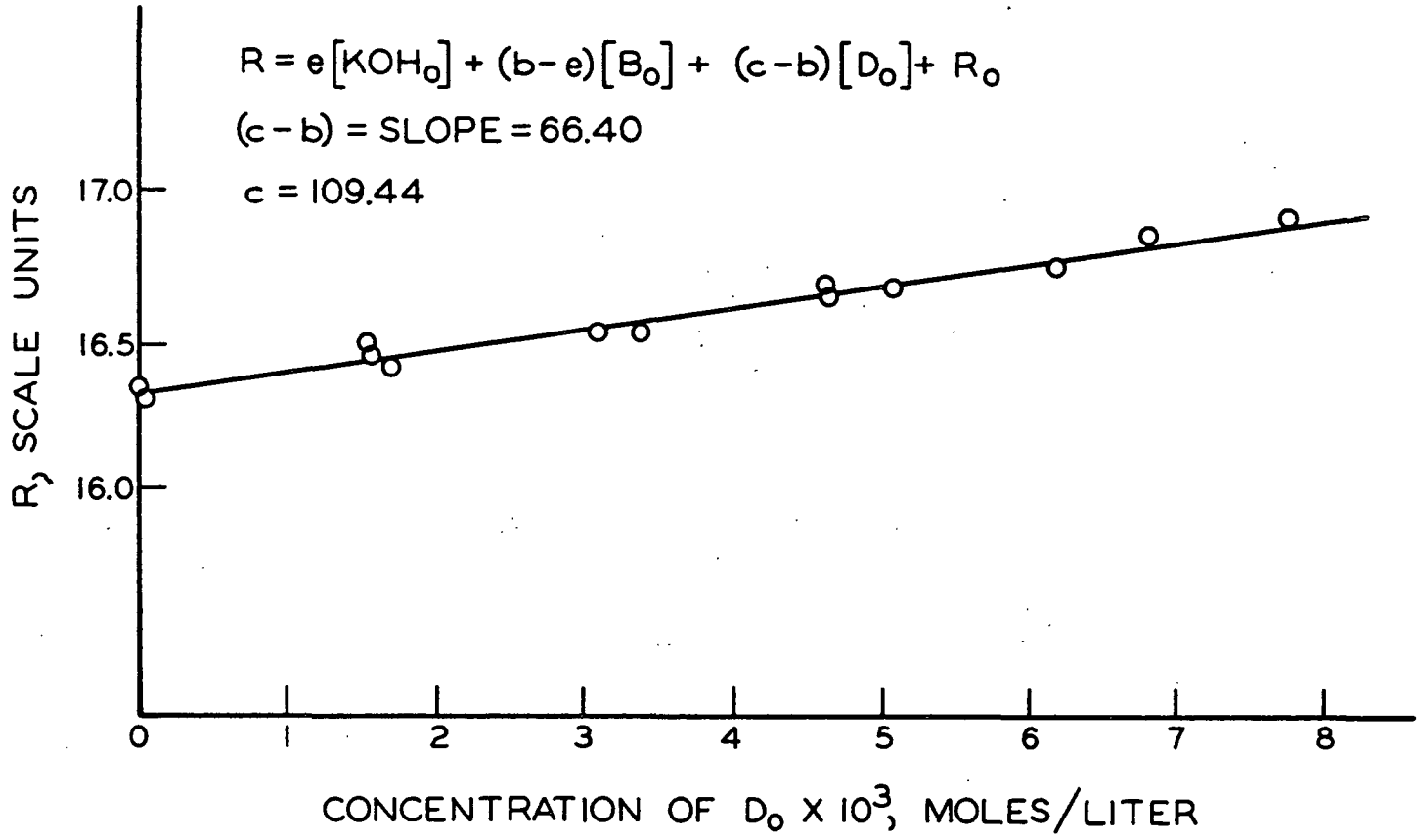


Figure 44. Determination of the Refractive Index Constant c for Methyl-α-D-galactopyranoside

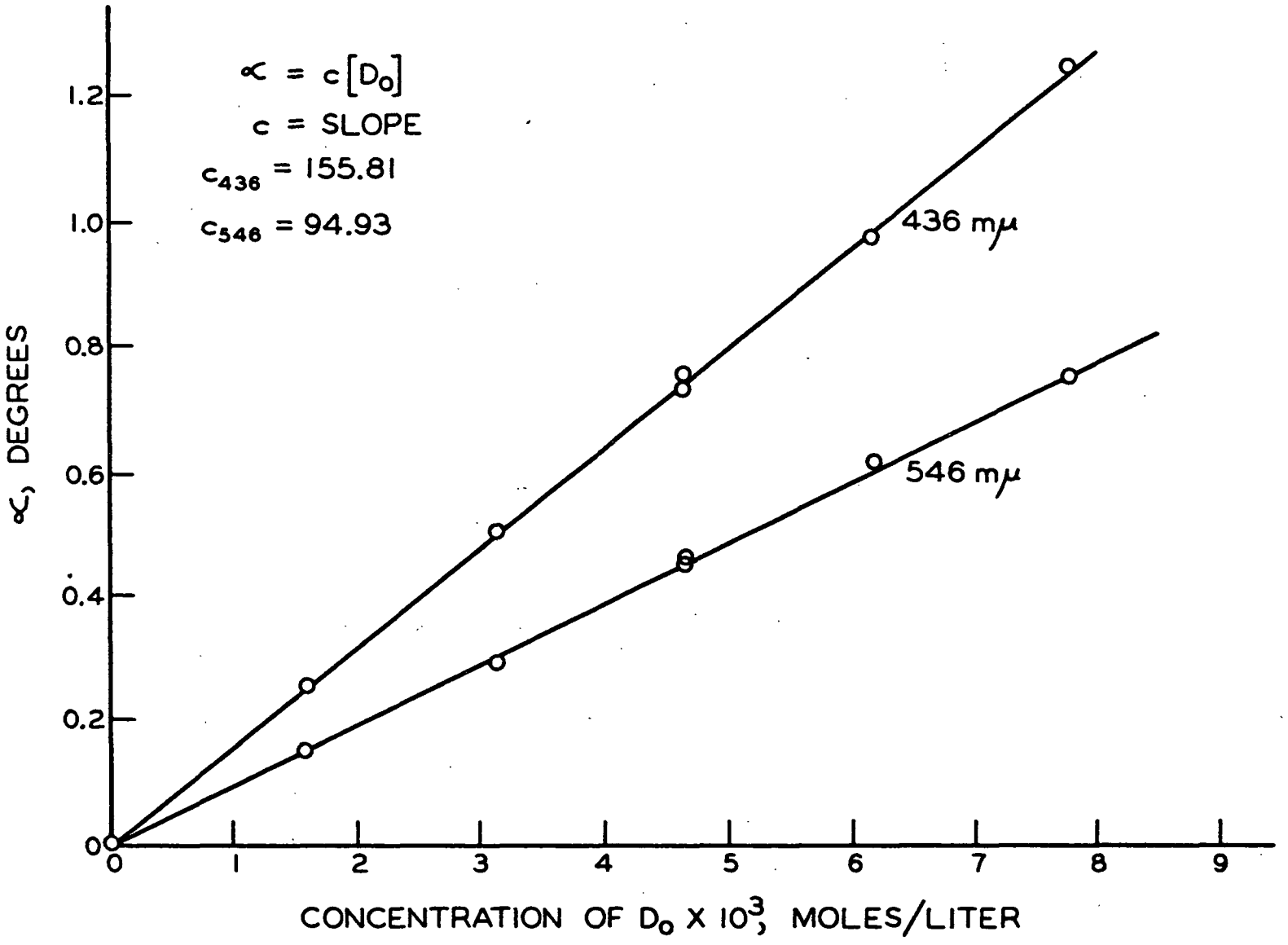


Figure 45. Determination of the Optical Rotation Constant c for Methyl-α-D-galactopyranoside

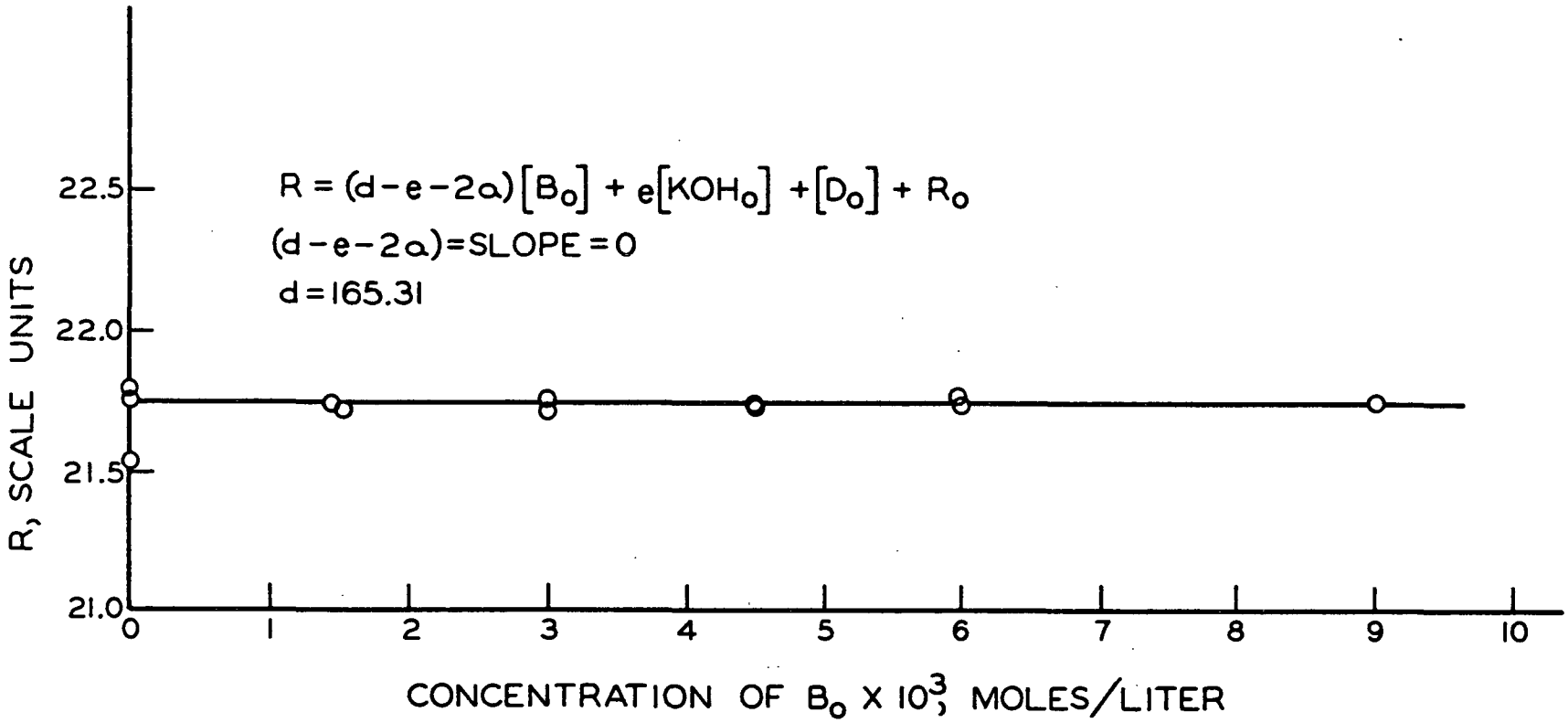


Figure 46. Determination of the Refractive Index Constant d for Methyl-α-D-galactopyranoside

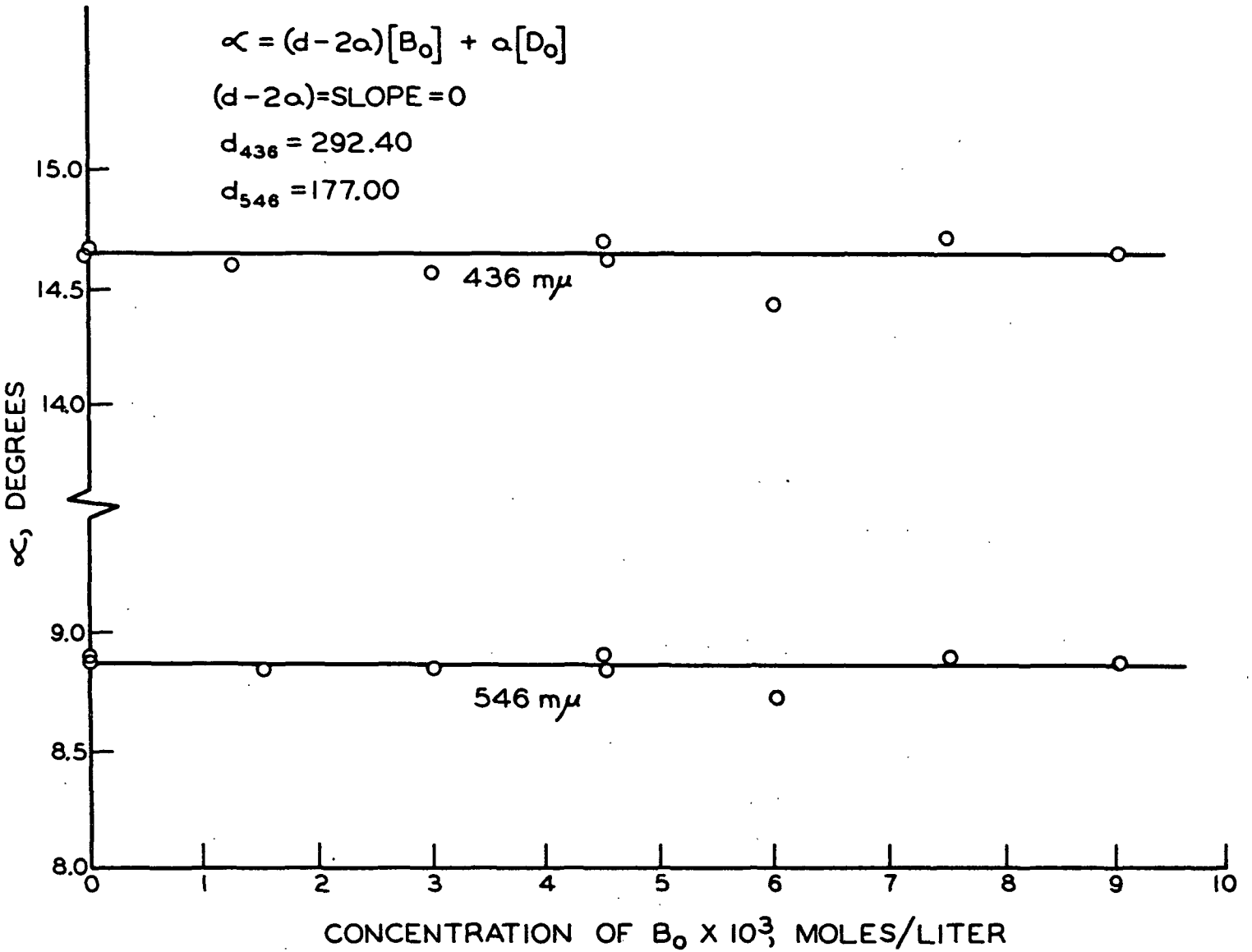


Figure 47. Determination of the Optical Rotation Constant d for Methyl-α-D-galactopyranoside

During the determinations of the $\underline{c}$ constant for refractive index, two points were run with $[\underline{B}_o]$ increased to 0.200 mole/liter. Because more KOH was necessary to maintain an excess of alkali, the ionic strength of the solution also increased. The resulting slope did not agree with that at a $[\underline{B}_o]$ value of 0.100 mole/liter; neither was the intercept as predicted. In both cases, the excess of $\underline{B}_o$ was enough to ensure that the equilibria were pushed far toward the BD complex, and it appears that ionic strength affects the numerical values of the constants, in addition to the usual equilibrium effects.

VERIFICATION OF RESULTS

The $\Delta\alpha_{436}$ data were used to calculate [BD] values and were found to check within 0.003 mole/liter. Because the optical rotation results do not contain a term for $[BD_2]$, the accuracy of the concentration determinations can be checked. When Equation (24) is solved for [BD] at each wavelength, and the two equations are combined, the following expression for $[\underline{D}_o]$ is obtained:

$$[D_o] = 0.074\alpha_{436} - 0.111\alpha_{546} \qquad (94).$$

The values of $[\underline{D}_o]$ calculated from Equation (94) were compared with the actual experimental values of $[\underline{D}_o]$. The results are given in Table XIII. Although some variation occurred, the majority of the calculated values checked with the experimental values within a 3% difference. In general, the variation was well within the predicted $\pm$ 0.003 mole/liter variance.

The ratio test was used to check the BD coefficient of the $\Delta\underline{R}$ working equation. From Equations (56) and (58), if no BD_2 is present,

$$\Delta R/\Delta\alpha_{546} = -4.9/6.4 = -0.8 \qquad (95).$$

TABLE XIII

A COMPARISON OF EXPERIMENTAL AND CALCULATED $[\underline{D}_o]$ VALUES FROM OPTICAL ROTATION DATA

$[\underline{D}_o]$, exptl.	$[\underline{D}_o]$, calcd.	Difference	Difference, %
0.020	0.021	-0.001	5
0.030	0.031	-0.001	3
0.040	0.041	-0.001	2
0.050	0.050	-0.000	0
0.055	0.053	+0.002	4
0.060	0.061	-0.001	2
0.067	0.066	+0.001	1
0.070	0.068	+0.002	3
0.080	0.077	+0.003	4
	Average:	0.001	3

At a $[\underline{D}_o]$ value of 0.01 mole/liter,

$$\Delta R/\Delta\alpha_{546} = -0.3 \qquad (96).$$

Because the $\Delta\alpha_{546}$ data gave several independent checks, it can be assumed that if any error exists it is in the $\underline{\Delta R}$ coefficient. If the ratio of -0.3 is assumed correct, the $\underline{\Delta R}$ coefficient for BD_2 becomes approximately 2.0. From Fig. 10 it can be seen that the maximum concentration of BD_2 occurs at a $[\underline{D}_o]$ of 0.08 mole/liter. On calculation of $[BD_2]$ at this point, using 2.0 as the [BD] coefficient in the $\underline{\Delta R}$ working equation, a value of 0.008 mole/liter is obtained, compared to the previous value of 0.005 mole/liter. This is roughly within the expected error in $[BD_2]$. Therefore, although the $[BD_2]$ values may be somewhat low, they are not grossly incorrect and can be used as is. In any case, the concentration

of BD_2 will always be much less than that of BD. In general, the concentration of the complexes and the stability constants for the several equilibria were obtained with sufficient accuracy for a reliable comparison between the model compounds.

METHYL-α-D-MANNOPYRANOSIDE

DETERMINATION OF CONSTANTS

The constants were determined in the same manner as with the α-galactoside, using refractive index and optical rotation measurements. The results are presented in Fig. 48-53. The $\underline{R}$ versus $[\underline{B}_o]$ (Fig. 52) relation did not give a significant F-test at the 95% level. However, the number of data points was small, and the statistics must be interpreted with due caution.

VERIFICATION OF RESULTS

The ratio test was applied to the $\Delta\alpha$ coefficients. If no BD is present, the working equations yield:

$$\Delta\alpha_{436}/\Delta\alpha_{546} = 13/10 = 1.3 \qquad (97).$$

From the actual values at $[\underline{D}_o]$ of 0.09 mole/liter,

$$\Delta\alpha_{436}/\Delta\alpha_{546} = 1.6 \qquad (98).$$

If no BD_2 is present, the equations give

$$\Delta\alpha_{436}/\Delta\alpha_{546} = 1.7 \qquad (99).$$

From the actual values of $[\underline{D}_o]$ equals 0.01 mole/liter,

$$\Delta\alpha_{436}/\Delta\alpha_{546} = 1.6 \qquad (100).$$

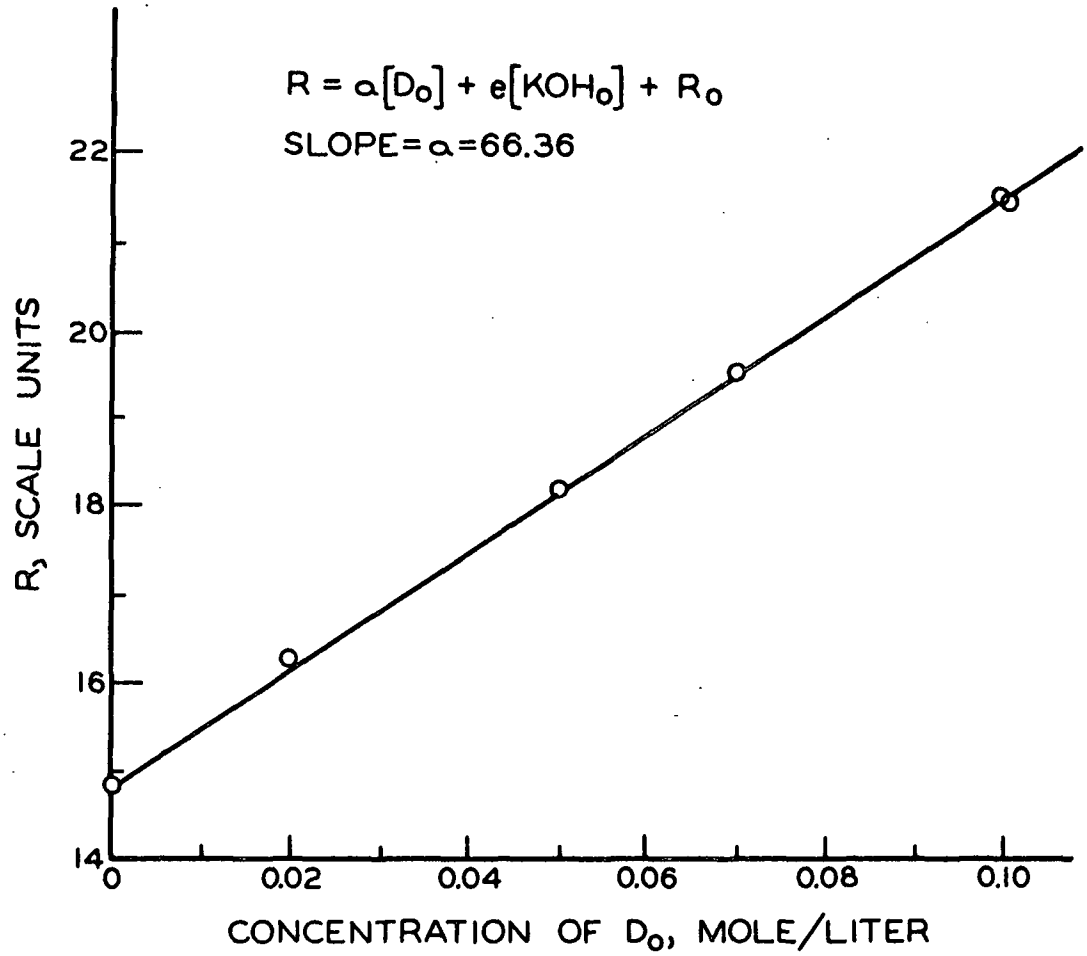


Figure 48. Determination of the Refractive Index Constant a for Methyl-α-D-mannopyranoside

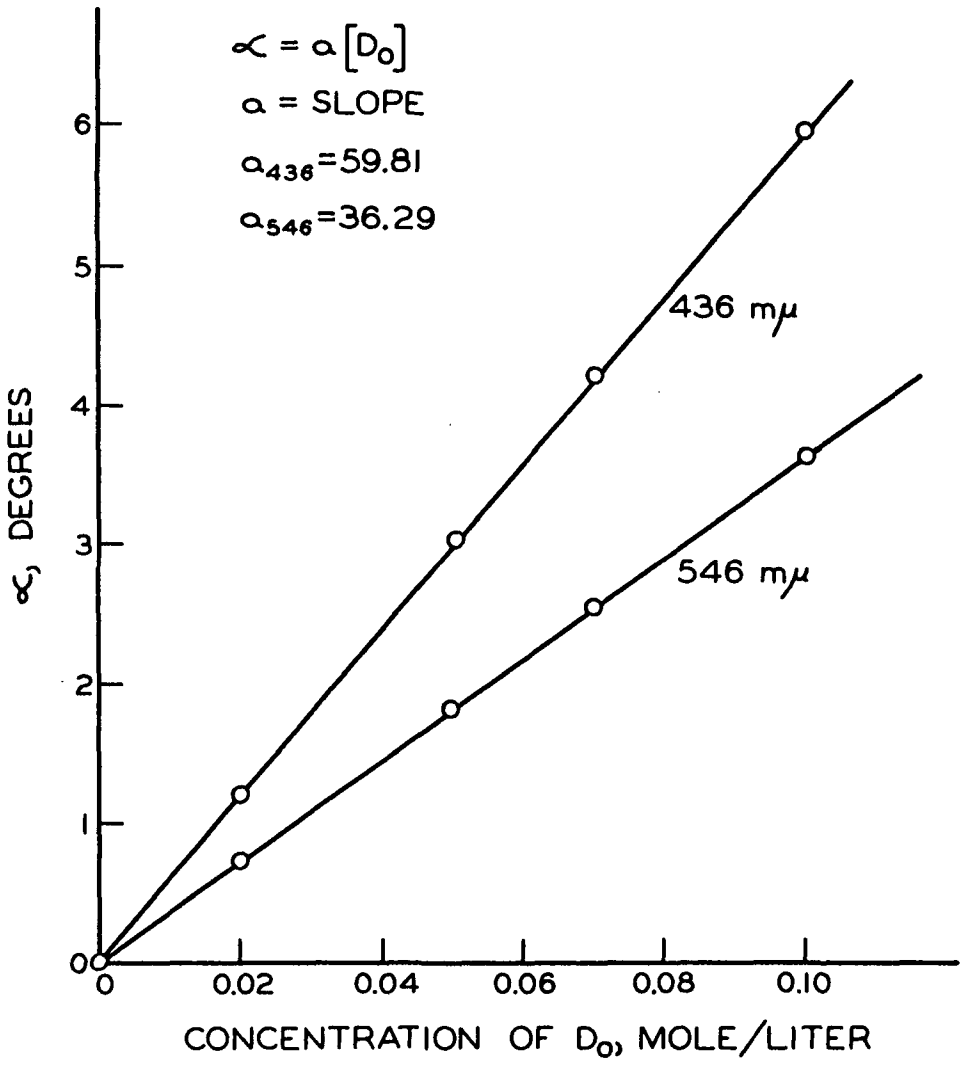


Figure 49. Determination of the Optical Rotation Constant a for Methyl-α-D-mannopyranoside

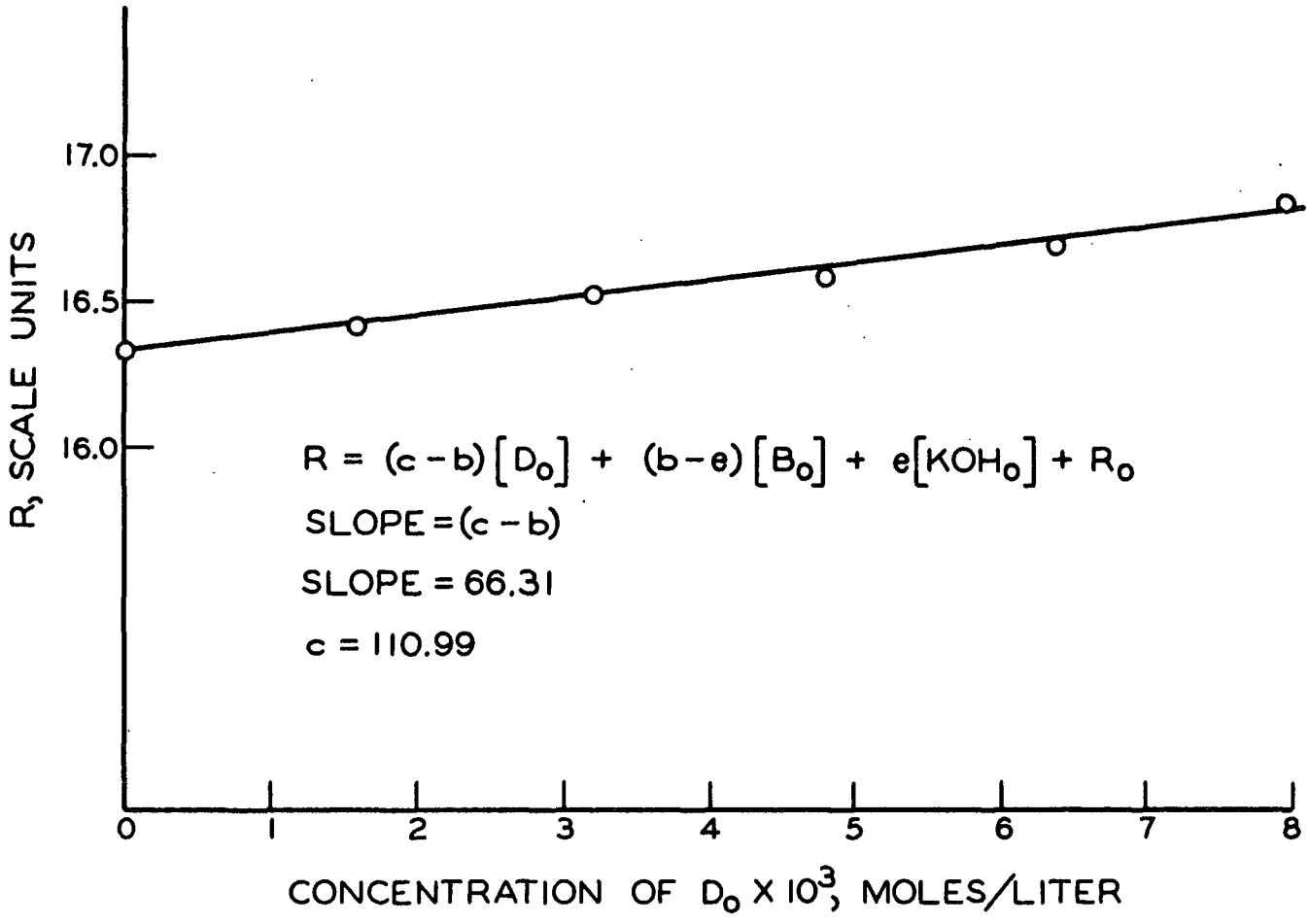


Figure 50. Determination of the Refractive Index Constant c for Methyl-α-D-mannopyranoside

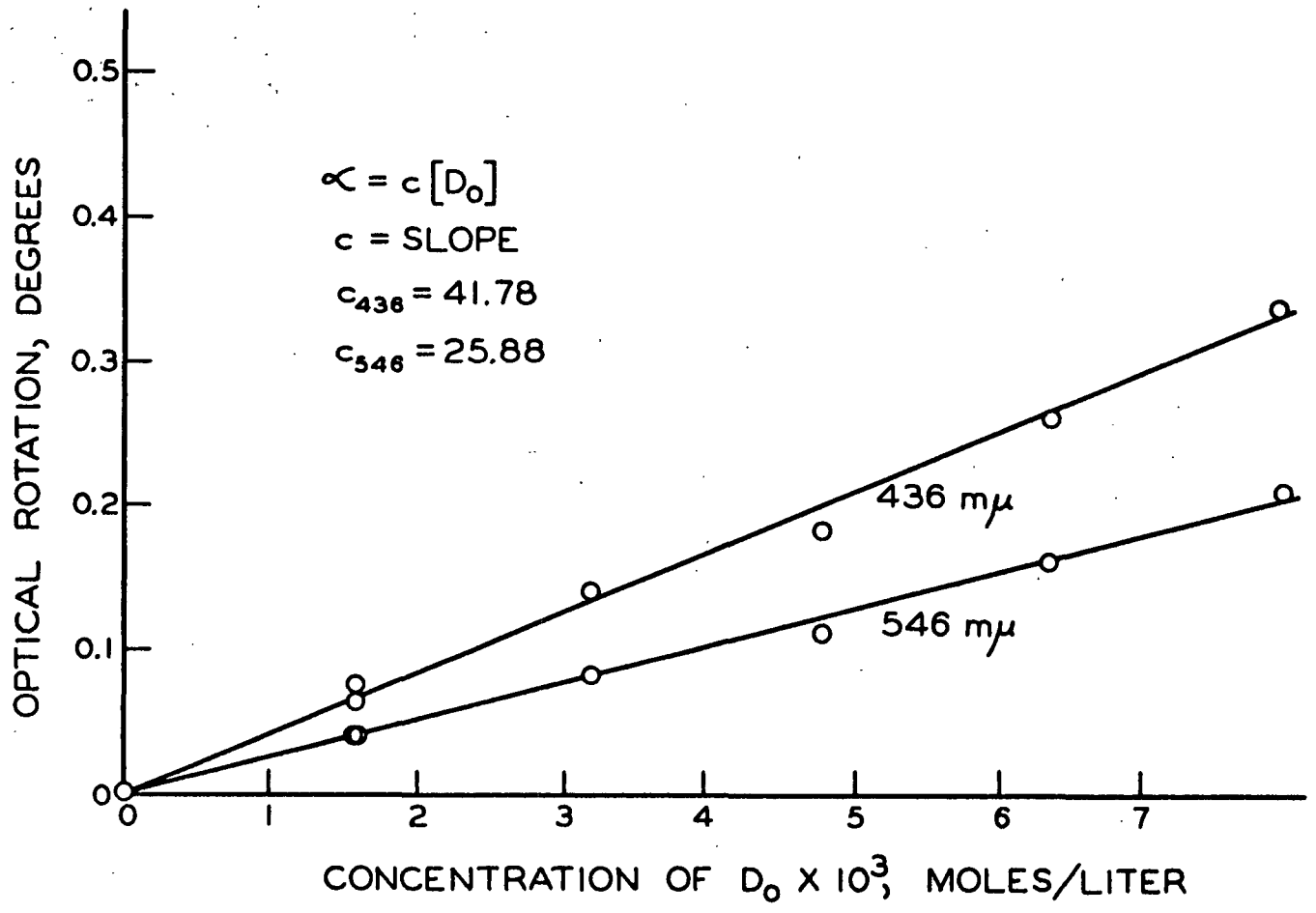


Figure 51. Determination of the Optical Rotation Constants c for Methyl-α-D-mannopyranoside

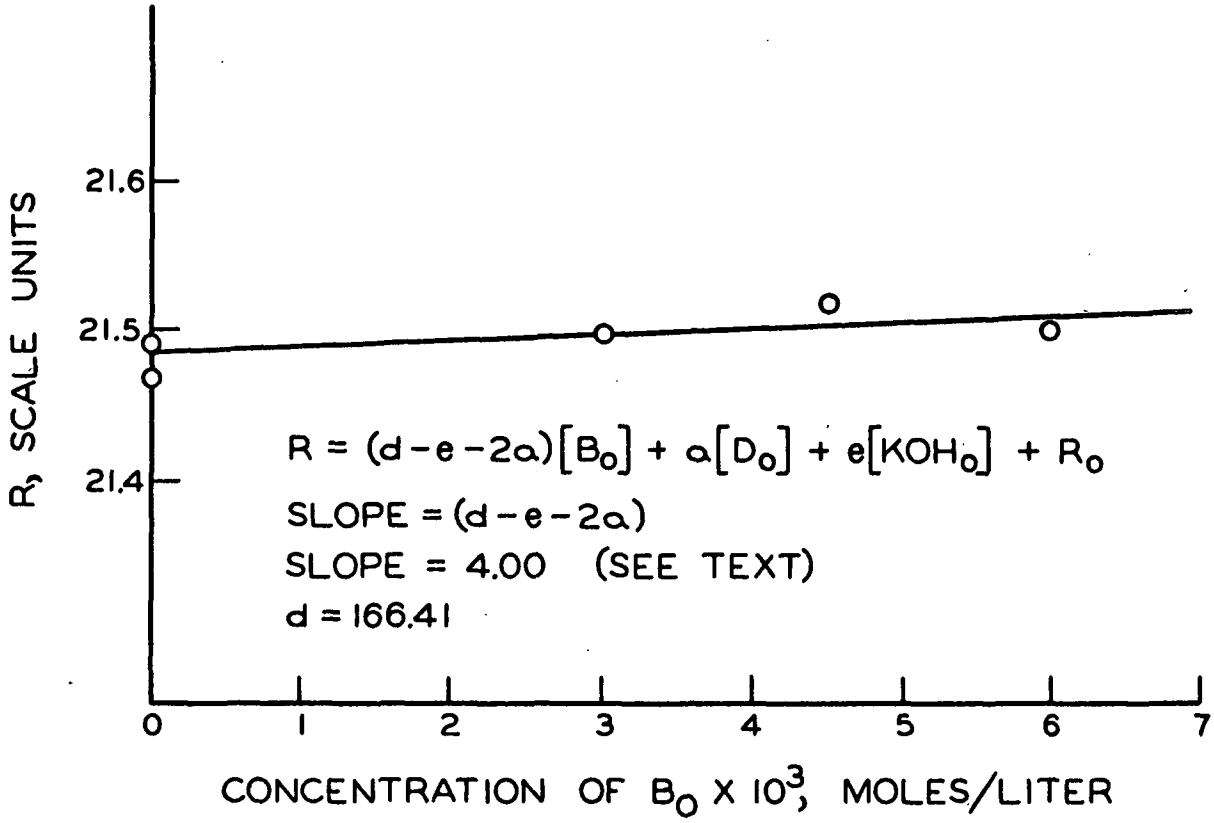


Figure 52. Determination of the Refractive Index Constant d for Methyl-α-D-mannopyranoside

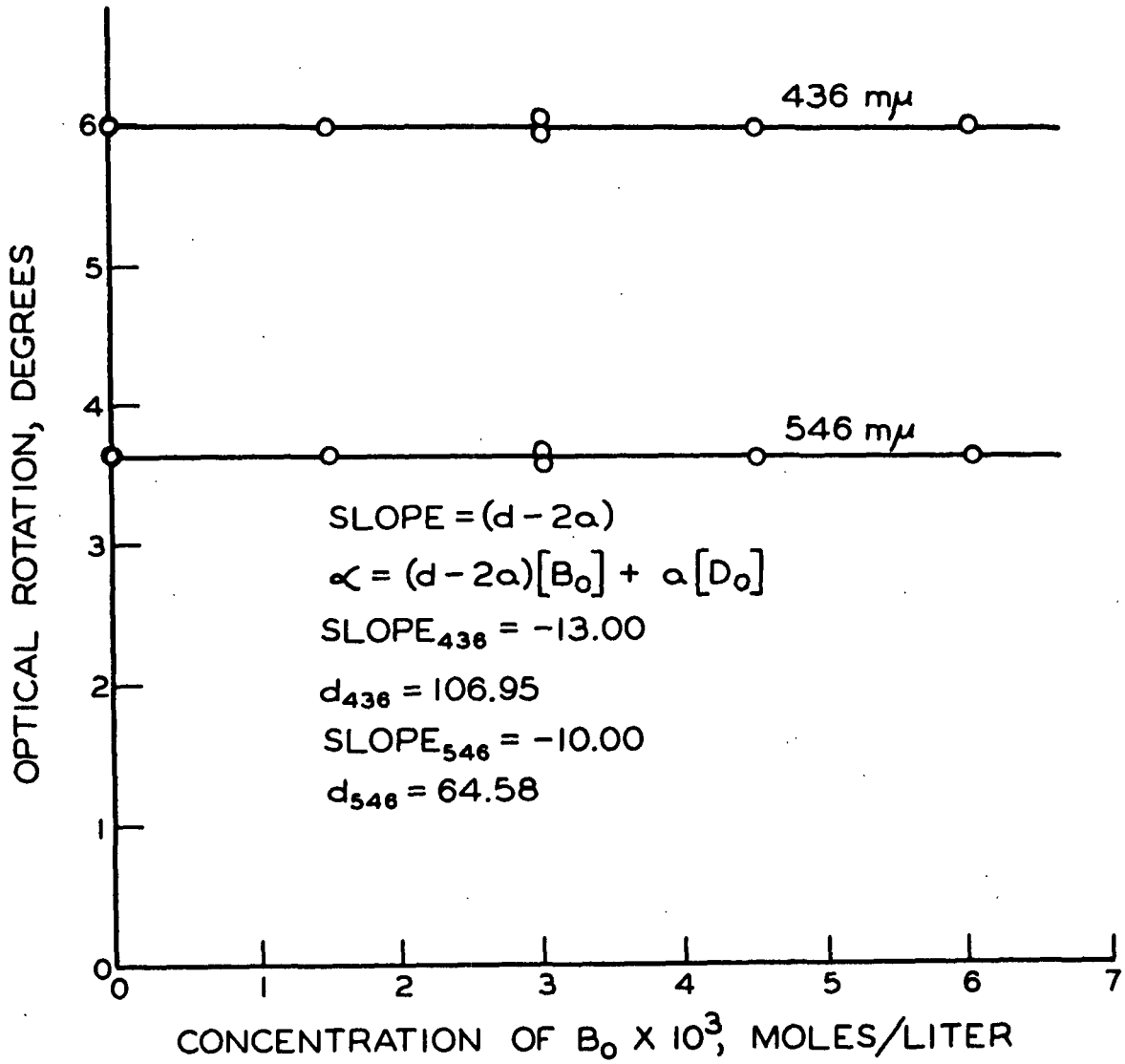


Figure 53. Determination of the Optical Rotation Constants d for Methyl-α-D-mannopyranoside

The ratio of the coefficients checked rather closely with the ratio given by the actual values. This serves to confirm the reliability of the optical rotation data.

An attempt was made to check the coefficients of the $\Delta\underline{R}$ working equation in the hope of obtaining some correction factor. However, as the $\Delta\underline{R}$ values at the ends of the curve were of little value, no correction could be determined.

METHYL-4-O-METHYL-α-D-MANNOPYRANOSIDE

DETERMINATION OF CONSTANTS

The necessary constants were determined by refractive index and optical rotation measurements and are presented in Fig. 54-59. Only the [D] versus $\underline{R}$, [:D] versus α, and [BD] versus α data gave a significant F-test at the 95% level. However, the $[BD_2]$ curves had insufficient data points for reliable statistics, and the ratio test for the resulting coefficients showed the reliability of the coefficients. The [BD] versus $\underline{R}$ curve had a slope close to zero, which seemed unusual in comparison to the other model compounds, and must be used with caution.

VERIFICATION OF RESULTS

The ratio test was used to check the $\Delta\alpha$ coefficients. If no BD is present, the working equations give

$$\Delta\alpha_{436}/\Delta\alpha_{546} = 20/11 = 1.82 \qquad (101).$$

At $[\underline{D}_o]$ equal to 0.09 mole/liter, the value is

$$\Delta\alpha_{436}/\Delta\alpha_{546} = 1.81 \qquad (102).$$

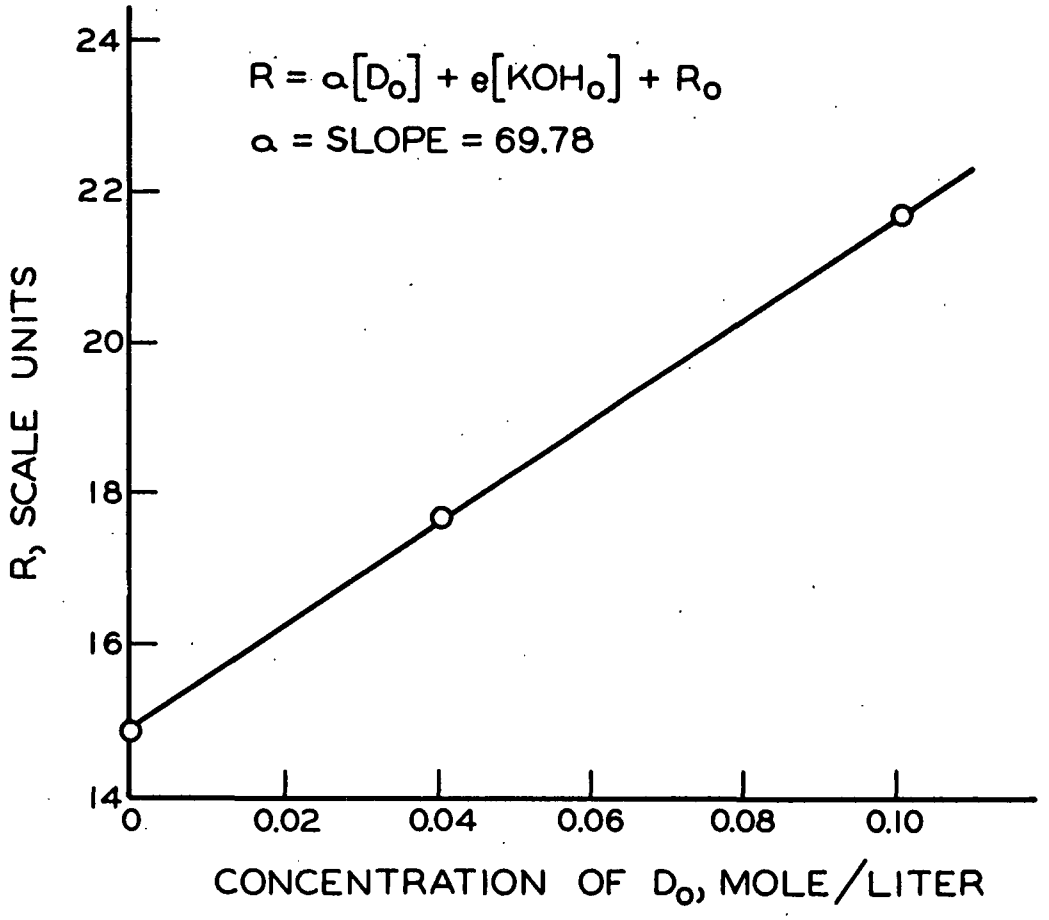


Figure 54. Determination of the Refractive Index Constant a for Methyl-4-O-methyl-α-D-mannopyranoside

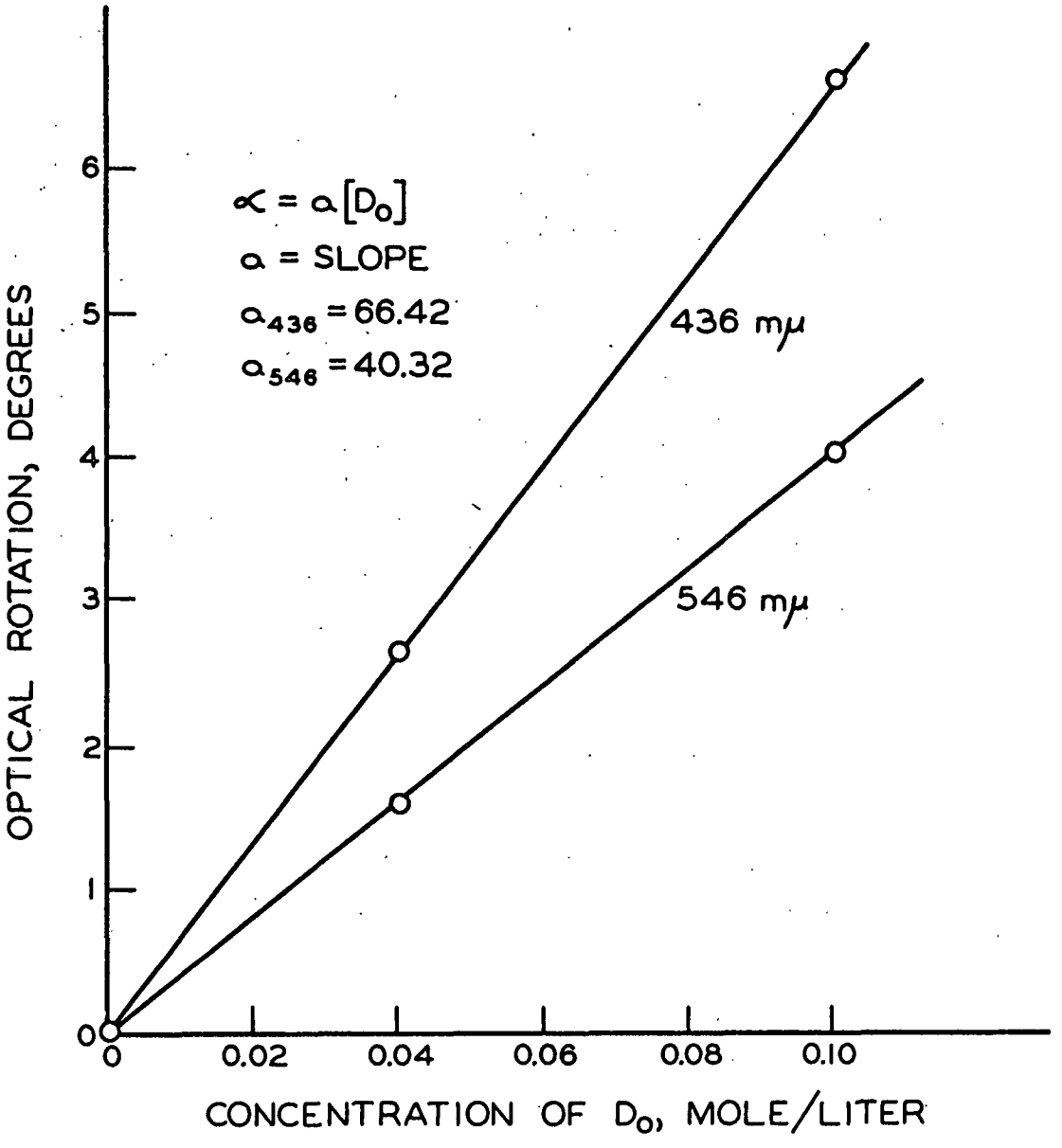


Figure 55. Determination of the Optical Rotation Constant a for Methyl-4-O-methyl-α-D-mannopyranoside

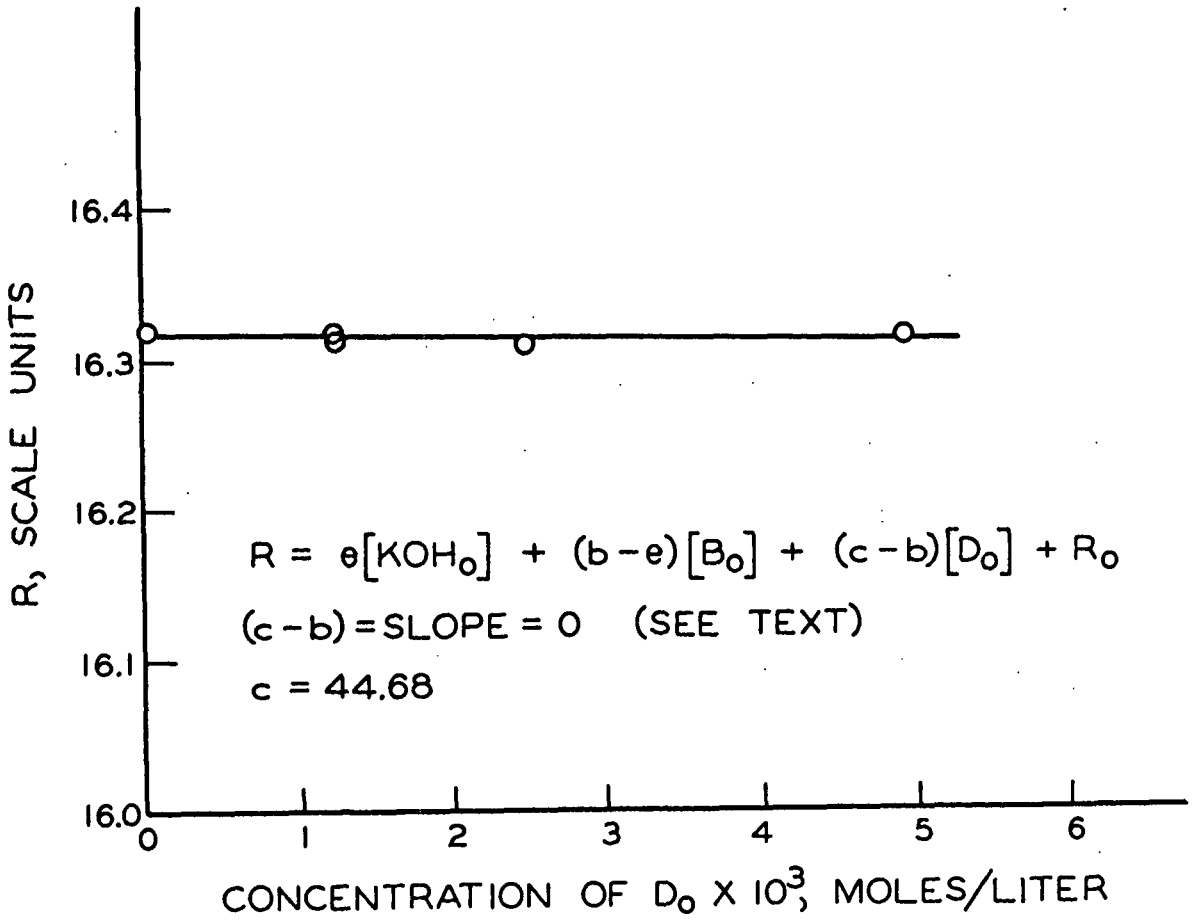


Figure 56. Determination of the Refractive Index Constant c for Methyl-4-O-methyl-α-D-mannopyranoside

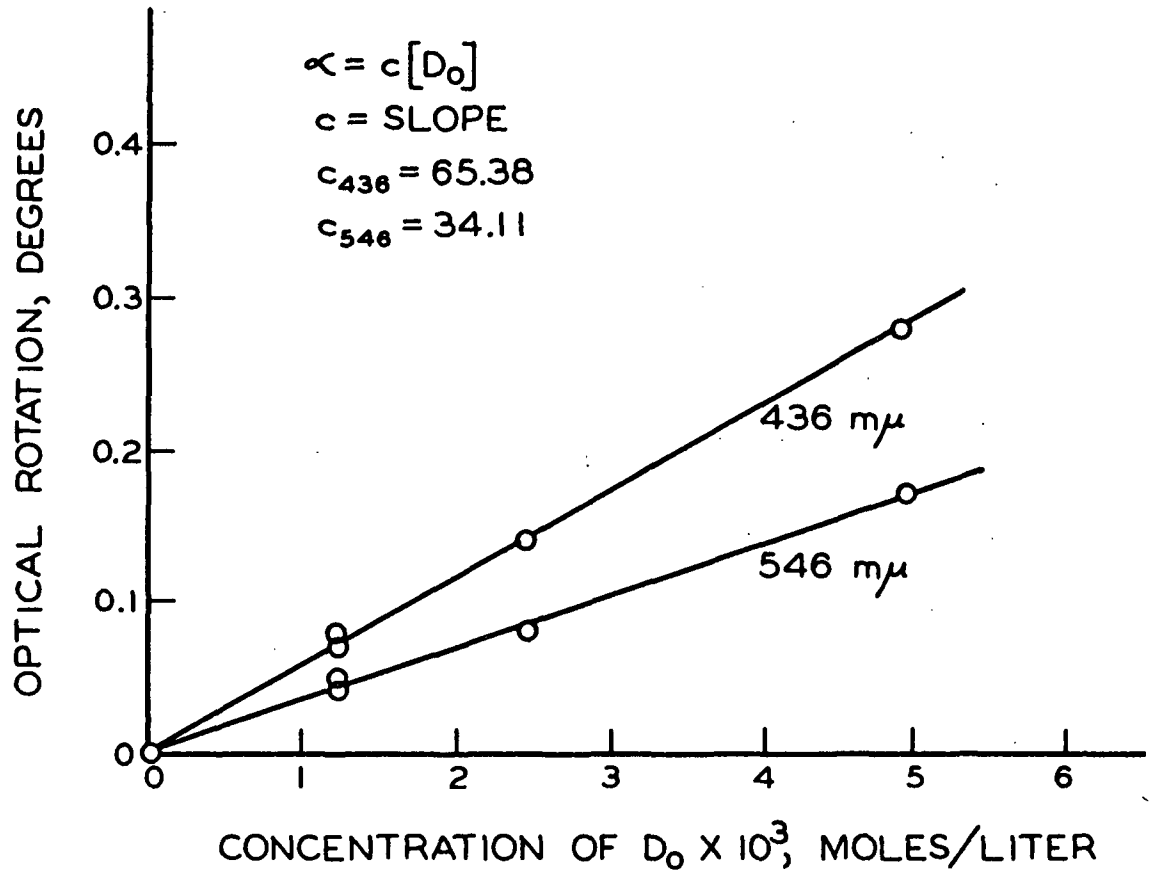


Figure 57. Determination of the Optical Rotation Constants c for Methyl-4-O-methyl-α-D-mannopyranoside

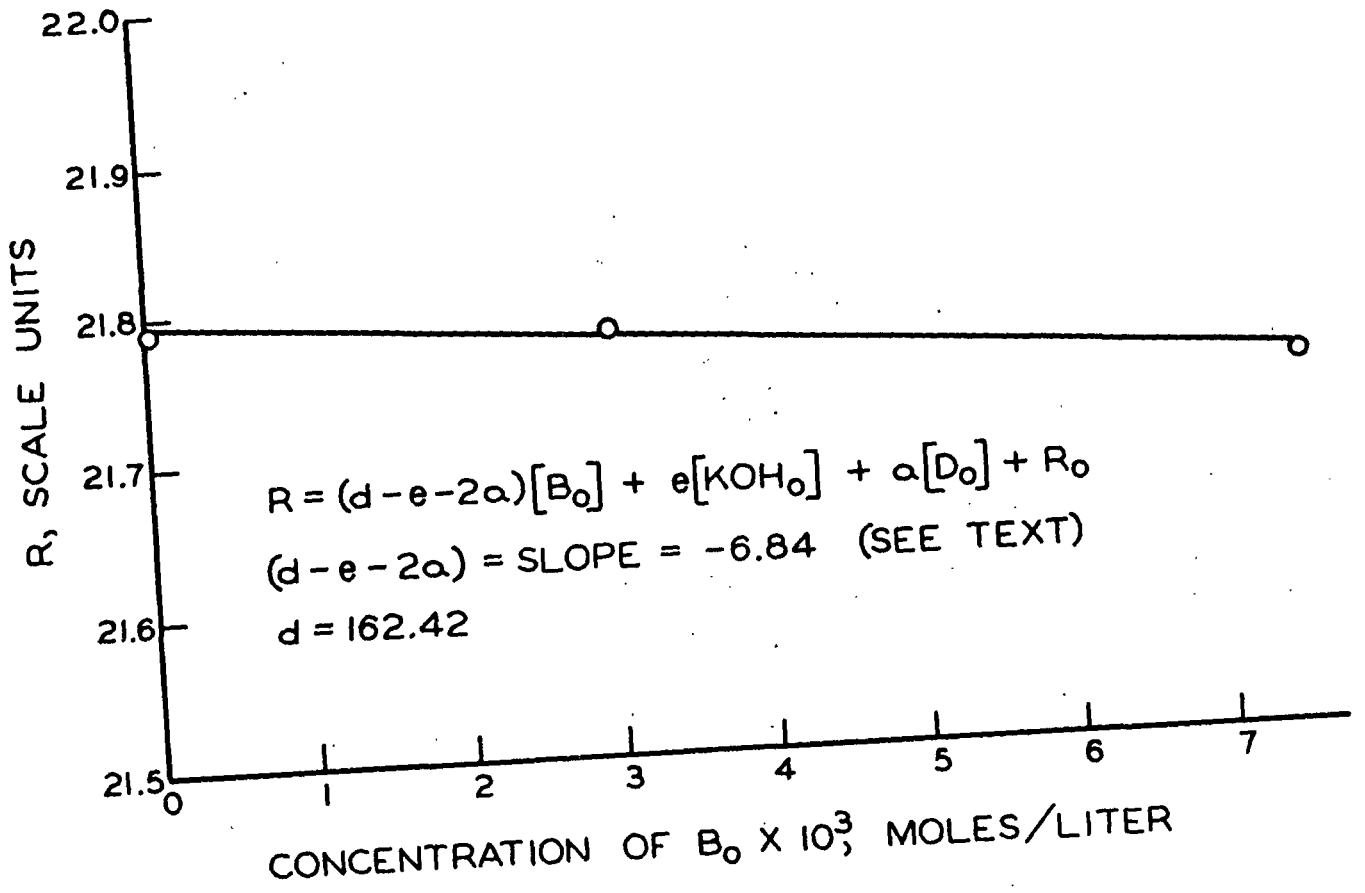


Figure 58. Determination of the Refractive Index Constant d for Methyl-4-O-methyl-α-D-mannopyranoside

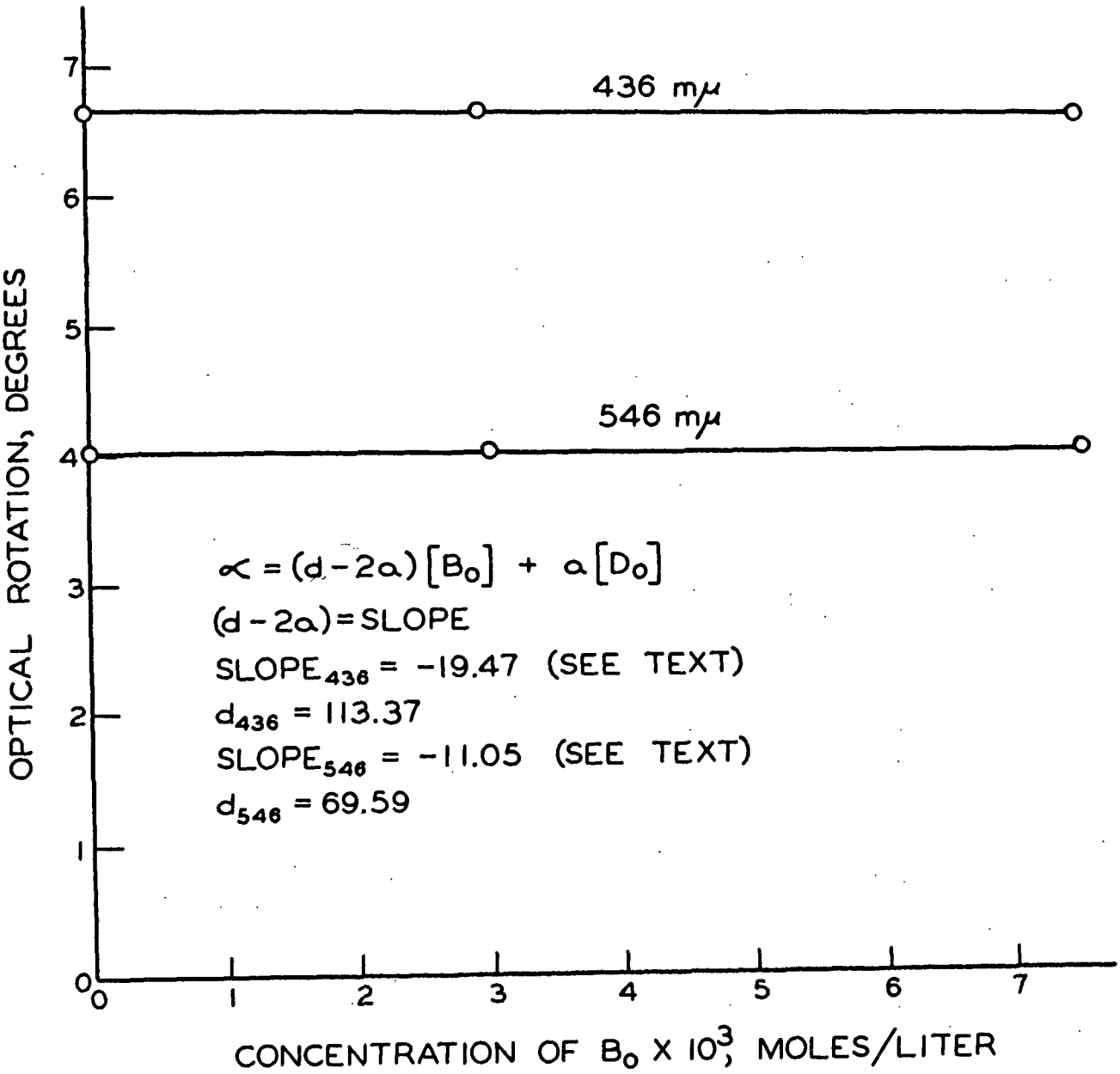


Figure 59. Determination of Optical Rotation Constants d for Methyl-4-O-methyl-α-D-mannopyranoside

If no BD_2 is present, the working equations give

$$\Delta\alpha_{436}/\Delta\alpha_{546} = 10/6.2 = 1.61 \qquad (103).$$

From the data points at $[\underline{D}_o]$ equal to 0.01 mole/liter,

$$\Delta\alpha_{436}/\Delta\alpha_{546} = 1.61 \qquad (104).$$

Both the coefficients gave excellent checks. This was especially important with the BD_2 coefficients as they were calculated from slopes obtained with a minimum of data points.

The ratio test was also used on the $\Delta\underline{R}$ working equation. In the $\Delta\underline{R}$ equation the $[BD_2]$ coefficient should be reasonably accurate, while the [BD] coefficient not only seems too large, but was calculated from a slope that was in some doubt. Therefore, a corrected value for the [BD] coefficient was estimated by use of the ratio test. The value for the coefficient was set at 21.0, and the ratio was used as a test.

If no BD_2 is present, the new $\Delta\underline{R}$ equation gives the ratios of

$$\Delta\alpha_{436}/\Delta\underline{R} = 10/21 = 0.5 \quad \text{and} \quad \Delta\alpha_{546}/\Delta\underline{R} = 6.2/21 = 0.3$$

From the data at $[\underline{D}_o]$ equal to 0.01 mole/liter,

$$\Delta\alpha_{436}/\Delta\underline{R} = 0.5 \quad \text{and} \quad \Delta\alpha_{546}/\Delta\underline{R} = 0.3.$$

The new coefficient for [BD] in the $\Delta\underline{R}$ equation checked with both $\Delta\alpha$ values and should be close to the correct value.

It now remained to check the $[BD_2]$ coefficient in the $\Delta\underline{R}$ equation. If no BD is present, the working equation gives

$$\Delta\alpha_{436}/\Delta\underline{R} = 20/22 = 0.9 \quad \text{and} \quad \Delta\alpha_{546}/\Delta\underline{R} = 11/22 = 0.5.$$

From the data points at $[\underline{D}_o]$ equal to 0.09 mole/liter,

$$\Delta\alpha_{436}/\Delta\underline{R} = 0.7 \quad \text{and} \quad \Delta\alpha_{546}/\Delta\underline{R} = 0.4.$$

A reasonable check on the $[BD_2]$ coefficient was therefore obtained, and the corrected $\Delta\underline{R}$ equation takes the form

$$\Delta R = 21.0[BD] + 22.0[BD_2] \qquad (105).$$

The above equation was used to check the [BD] and $[BD_2]$ values obtained from the optical rotation data. Using Equation (105), $\Delta\underline{R}$ was calculated from the [BD] and $[BD_2]$ values, and the results compared to the $\Delta\underline{R}$ values from the polynomial in $\Delta\underline{R}$. As shown below, reasonable agreement was obtained.

$[\underline{D}_o]$	$\Delta R_{calcd.}$	$\Delta R_{polynomial}$
0.74	0.49	0.42
0.05	0.54	0.48
0.06	0.52	0.52

This also served as a check on the [BD] and $[BD_2]$ values of the 4-O-methyl-α-mannoside system.

METHYL-4-O-METHYL-β-D-MANNOPYRANOSIDE

DETERMINATION OF CONSTANTS

The fourth model compound studied was the methyl-4-O-methyl-β-mannoside. The constants were determined from refractive index and optical rotation readings, with the results shown in Fig. 60-64. In the case of the $[BD_2]$ curves, only two points were available. However, the ratio test gave good checks with the $[BD_2]$ coefficients.

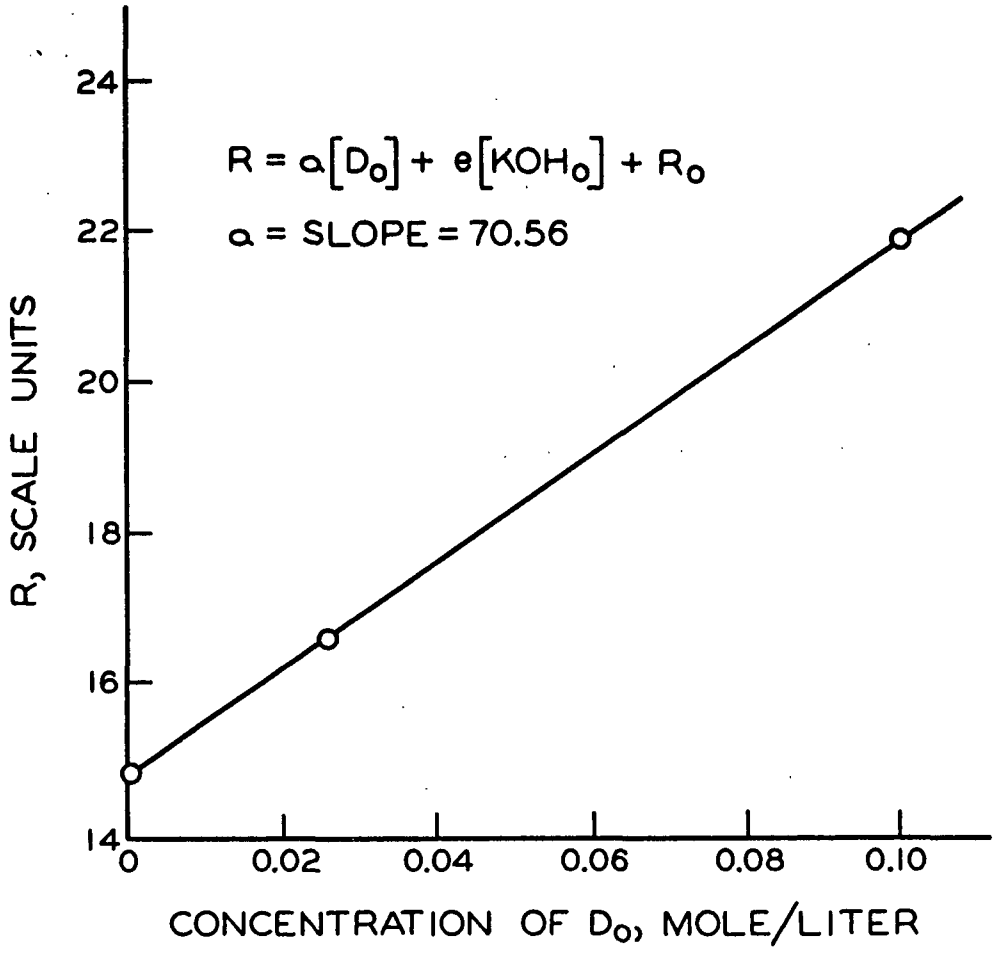


Figure 60. Determination of the Refractive Index Constant a for Methyl-4-O-methyl-β-D-mannopyranoside

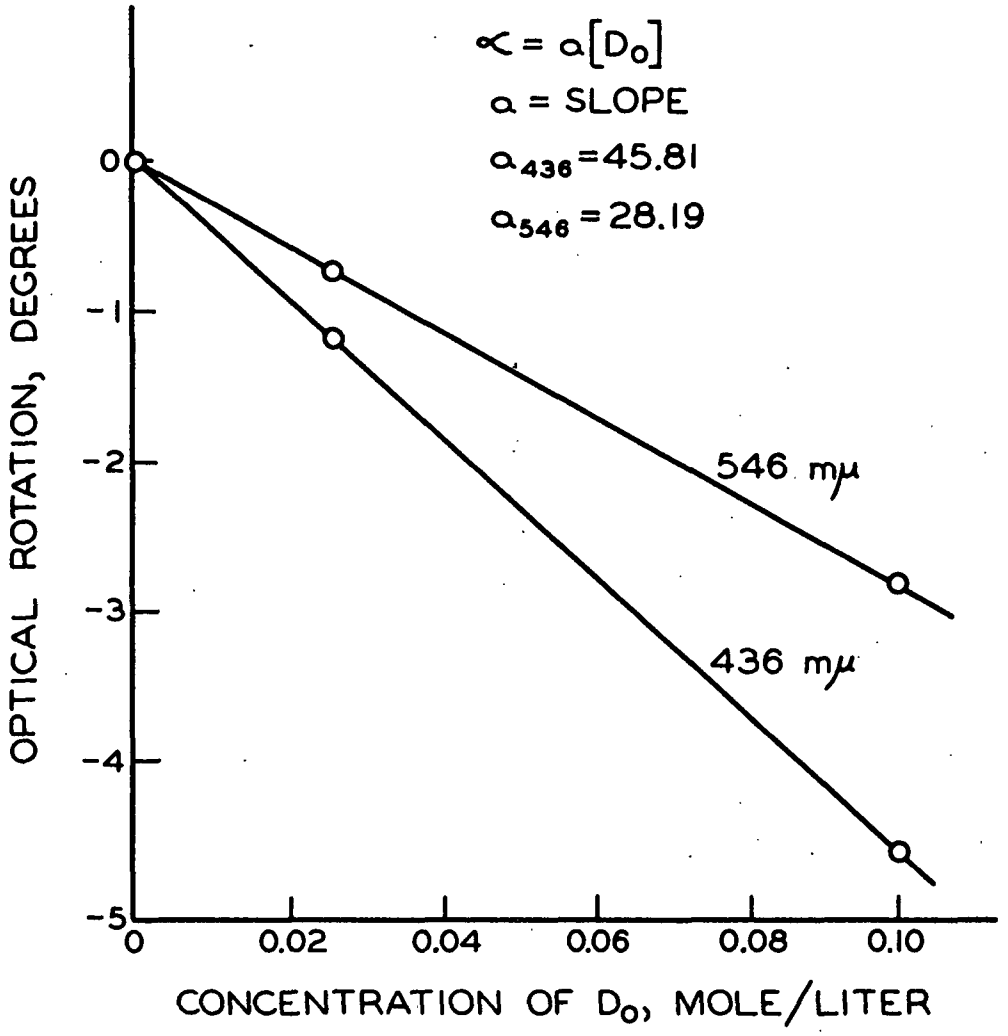


Figure 61. Determination of the Optical Rotation Constant a for Methyl-4-O-methyl-β-D-mannopyranoside

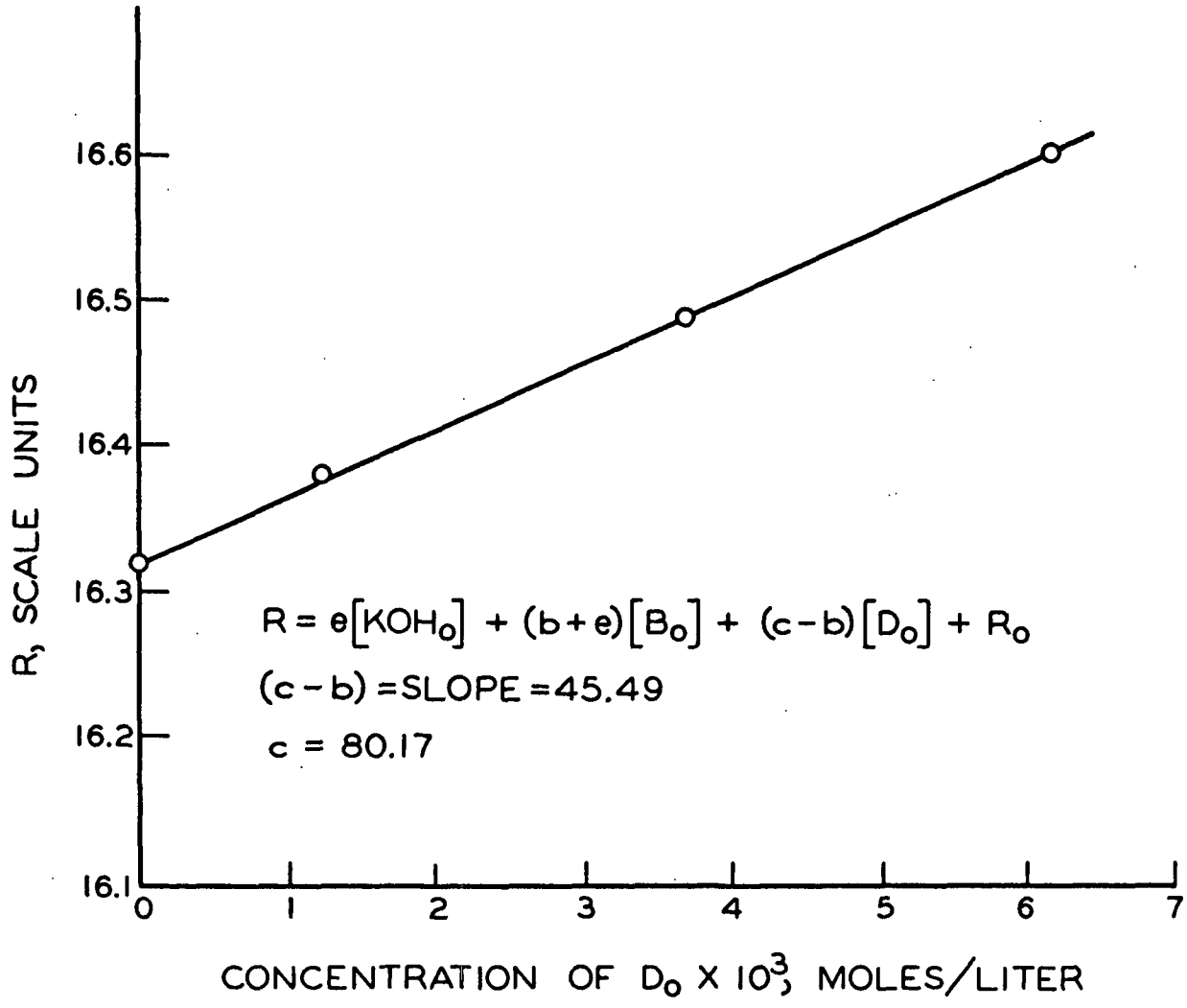


Figure 62. Determination of the Refractive Index Constant c for Methyl-4-O-methyl-β-D-mannopyranoside

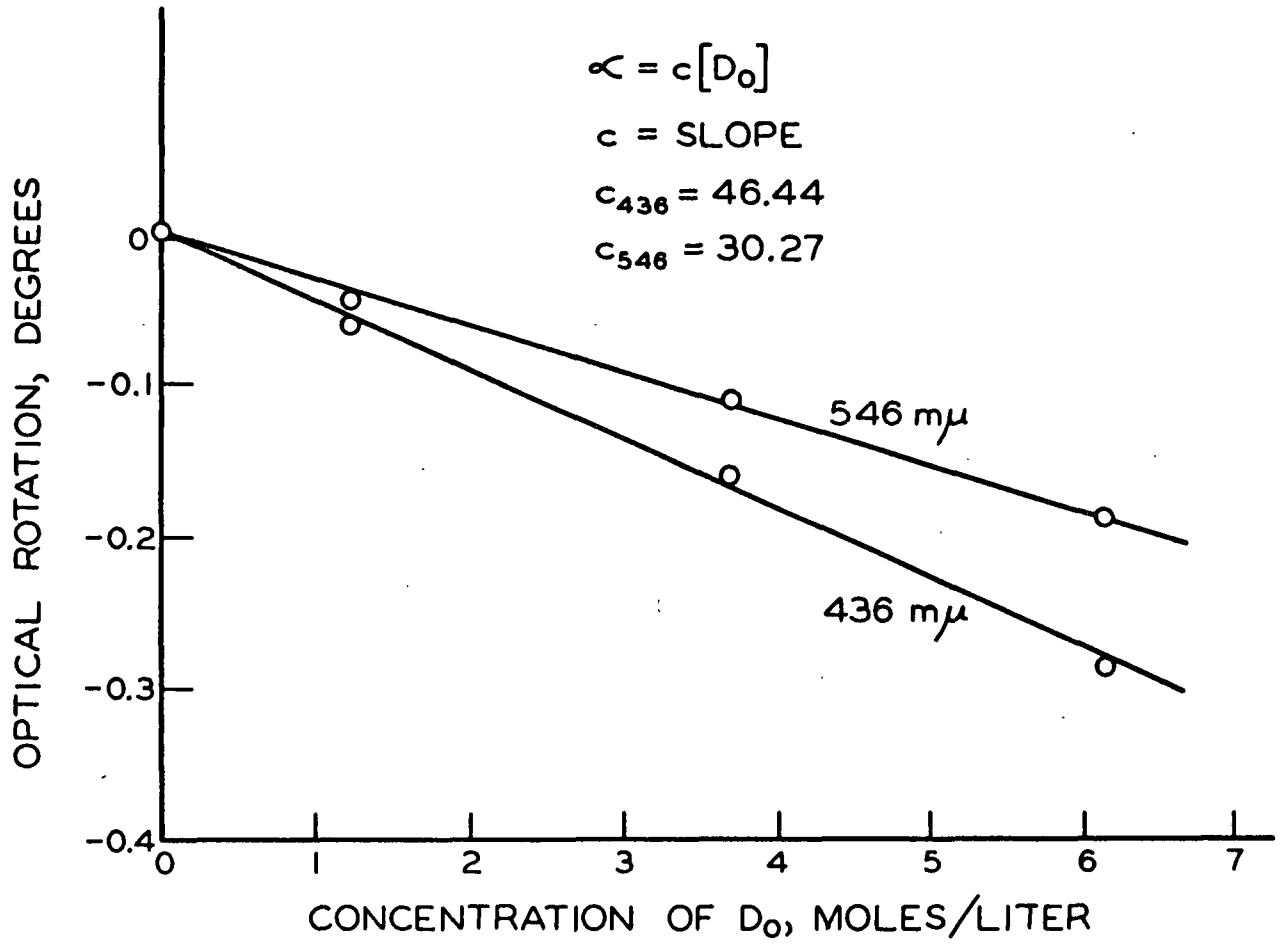


Figure 63. Determination of the Optical Rotation Constant c for Methyl-4-O-methyl-β-D-mannopyranoside

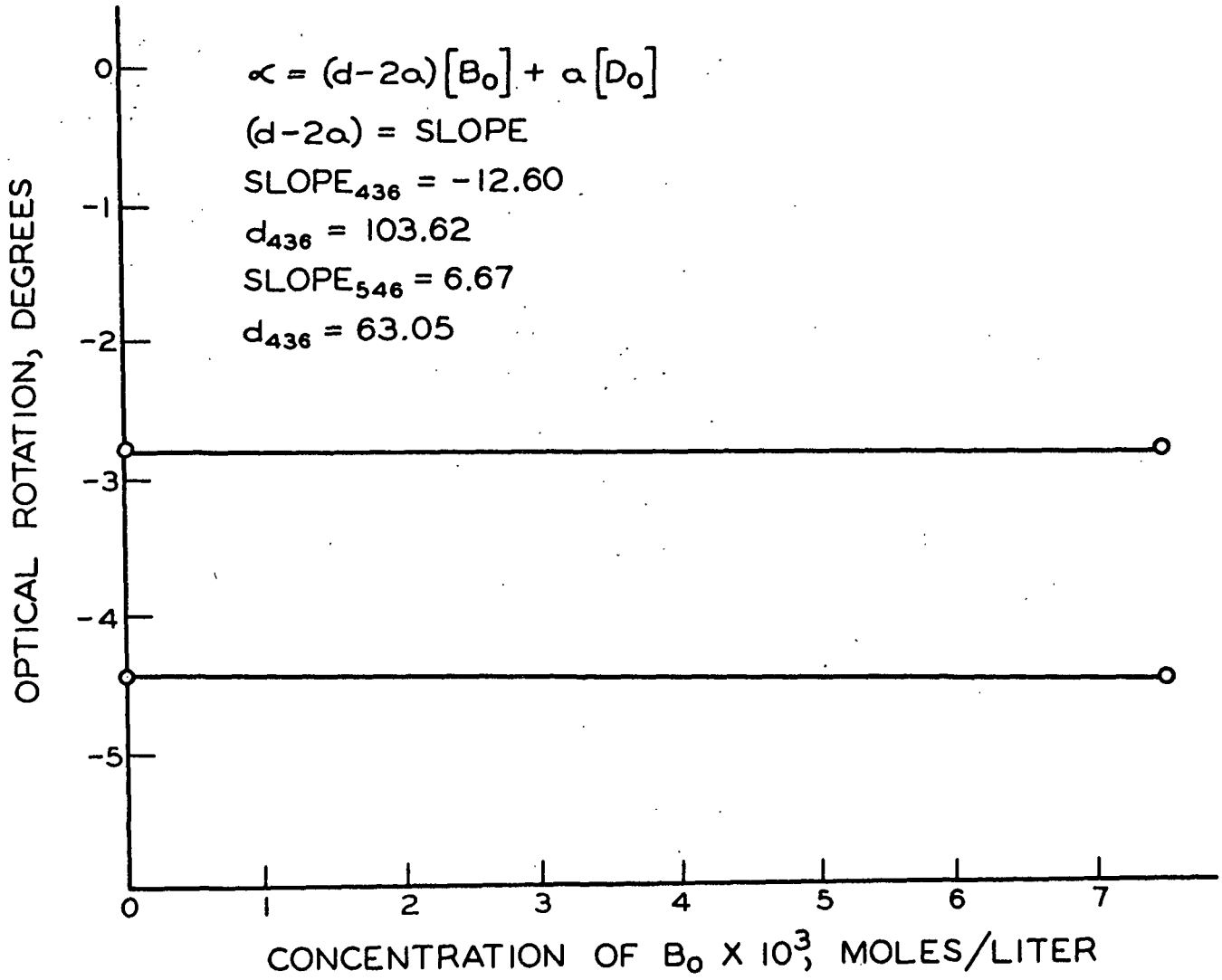


Figure 64. Determination of the Optical Rotation Constant d for Methyl-4-O-methyl-β-D-mannopyranoside

VERIFICATION OF RESULTS

The ratio test could not be used on the [BD] coefficient of the $\Delta\underline{R}$ equation as the $\Delta\alpha$ data points were near zero at $[\underline{D}_o]$ equal to 0.01 mole/liter. However, the ratio of $\Delta\alpha_{546}$ to $\Delta\underline{R}$ from the working equations was small, i.e., near 0.05, and since the ratio from the actual data will be zero, a rough check was obtained.

As the optical rotation values were not affected by the BD complex, the value of $[\underline{D}_o]$ could be back-calculated in a manner similar to that used with the α-galactoside. Table XIV gives the calculated and experimental values of $[\underline{D}_o]$, and it is apparent good agreement was obtained.

TABLE XIV

A COMPARISON OF EXPERIMENTAL AND CALCULATED $[\underline{D}_o]$ VALUES FROM OPTICAL ROTATION DATA

$[\underline{D}_o]_{exptl.}$, mole/liter	$[\underline{D}_o]_{calcd.}$, mole/liter	Difference, mole/liter	Difference, %
0.035	0.034	0.001	3
0.043	0.041	0.002	5
0.050	0.051	0.001	2
0.067	0.066	0.001	1
	Average:	0.001	3

APPENDIX III

TABULATION OF EXPERIMENTAL DATA

DETERMINATION OF REFRACTIVE INDEX CONSTANTS b and e

TABLE XV

DETERMINATION OF b

$[B_o]$, mole/liter[a]	R, scale units
0.0000	14.85
0.0207	15.17
0.0204	15.15
0.0210	15.16
0.0387	15.39
0.0393	15.38
0.0608	15.70
0.0597	15.70
0.0800	16.07
0.0800	16.03
0.0798	16.08
0.0999	16.32
0.1002	16.32
0.1020	16.38

[a] $[KOH_o]$ = 0.105 mole/liter.

TABLE XVI

DETERMINATION OF $\underline{e}$

[KOH], mole/liter	$\underline{R}$, scale units
0.0000	11.70
0.0200	12.39
0.0210	12.31
0.0400	12.92
0.0400	12.93
0.0600	13.56
0.0600	13.50
0.0800	14.10
0.1000	14.69
0.1000	14.66
0.1050	14.85
0.1050	14.83
0.1200	15.26

METHYL-α-D-GALACTOPYRANOSIDE DATA

TABLE XVII

DETERMINATION OF THE REFRACTIVE INDEX CONSTANTS [a]

$[D_o]$, mole/liter[a]	R, scale units
0.0000	14.82
0.0201	16.22
0.0300	17.00
0.0400	17.55
0.0502	18.19
0.0600	18.95
0.0798	20.13
0.1000	21.75
0.1000	21.79
0.1000	21.52

[a] $[KOH_o]$ = 0.105 mole/liter

TABLE XVIII

DETERMINATION OF THE OPTICAL ROTATION CONSTANT [a]

$[D_o]$, mole/liter[a]	α_{436}, °	α_{546}, °
0.0000	0.00	0.00
0.0201	2.95	1.80
0.0300	4.47	2.70
0.0501	7.35	4.46
0.0600	8.87	5.37
0.0798	11.59	7.02
0.1000	14.67	8.88
0.1000	14.66	8.88

[a] $[KOH_o]$ = 0.105 mole/liter

TABLE XIX

DETERMINATION OF THE REFRACTIVE INDEX CONSTANT c

$[D_o]$, mole/liter[a]	R, scale units
0.0000	16.32
0.0000	16.34
0.0016	16.49
0.0015	16.46
0.0017	16.40
0.0031	16.50
0.0034	16.50
0.0046	16.64
0.0046	16.69
0.0051	16.61
0.0062	16.72
0.0068	16.80
0.0077	16.89

[a] $[KOH_o]$ = 0.1050 mole/liter; $[B_o]$ = 0.1000 mole/liter.

TABLE XX

DETERMINATION OF THE OPTICAL ROTATION CONSTANT c

$[D_o]$, mole/liter[a]	α_{436}, °	α_{546}, °
0.0000	0.00	0.00
0.0015	0.28	0.17
0.0031	0.50	0.29
0.0046	0.75	0.45
0.0046	0.72	0.45
0.0062	0.97	0.61
0.0077	1.24	0.74

[a] $[KOH_o]$ = 0.105 mole/liter; $[B_o]$ = 0.100 mole/liter.

TABLE XXI

DETERMINATION OF THE REFRACTIVE INDEX CONSTANT $\underline{d}$

$[\underline{B}_o]$, mole/liter[a]	$\underline{R}$, scale units
0.0000	21.75
0.0012	21.72
0.0015	21.71
0.0030	21.73
0.0030	21.73
0.0030	21.69
0.0045	21.71
0.0045	21.71
0.0060	21.73
0.0060	21.70
0.0090	21.71

[a] $[KOH_o]$ = 0.1050 mole/liter; $[\underline{D}_o]$ = 0.1000 mole/liter.

TABLE XXII

DETERMINATION OF THE OPTICAL ROTATION CONSTANT $\underline{d}$

$[\underline{D}_o]$, mole/liter[a]	α_{436}, °	α_{546}, °
0.0000	14.66	8.88
0.0000	14.67	8.88
0.0015	14.59	8.84
0.0030	14.56	8.84
0.0045	14.61	8.86
0.0045	14.72	8.92
0.0060	14.40	8.72
0.0075	14.71	8.90
0.0090	14.63	8.87

[a] $[KOH_o]$ = 0.1050 mole/liter; $[\underline{D}_o]$ = 0.100 mole/liter.

TABLE XXIII

DETERMINATION OF ΔR VALUES

$[D_o]$, mole/liter[a]	R_{exp}, scale units	R_{nc}, scale units	ΔR_{av}, scale units
0.0000	16.32	16.32	0.00
0.0100	16.78	16.86	0.03
0.0100	16.87		
0.0200	17.39	17.41	0.05
	17.33		
	17.35		
0.0300	17.90	17.95	0.06
	17.88		
0.0400	18.38	18.49	0.14
	18.32		
0.0500	18.89	19.03	0.15
	18.86		
0.0550	19.18	19.30	0.18
	19.11		
	19.12		
0.0600	19.31	19.58	0.22
	19.40		
0.0667	19.77	19.94	0.16
	19.79		
0.0700	19.95	20.12	0.17
	19.94		
0.0800	20.52	20.66	0.16
	20.45		
0.0900	21.08	21.20	0.12
0.1000	21.79	21.75	-0.02
	21.75		

[a] $[KOH_o] = 0.105$ mole/liter; $[D_o] + [B_o] = 0.1000$ mole/liter.

TABLE XXIV

DETERMINATION OF $\Delta\alpha$ VALUES

$[\underline{D}_o]$, mole/liter[a]	$\alpha_{436,exp}$	$\alpha_{436,\underline{nc}}$	$\Delta\alpha_{436}$	$\alpha_{546,exp}$	$\alpha_{546,\underline{nc}}$	$\Delta\alpha_{546}$[b]
0.0000	0.00	0.00	0.00	0.00	0.00	0.00
0.0200	3.10	2.92	-0.18	1.87	1.77	-0.10
0.0300	4.66	4.39	-0.27	2.81	2.66	-0.15
0.0400	6.13	5.85	-0.28	3.71	3.54	-0.17
0.0500	7.62	7.31	-0.31	4.62	4.44	-0.18
0.0539	8.19	7.88	-0.31	4.95	4.77	-0.18
0.0550	8.30	8.04	-0.26	5.04	4.88	-0.16
0.0600	9.11	8.77	-0.34	5.51	5.31	-0.20
0.0667	10.06	9.75	-0.31	6.09	5.90	-0.19
0.0700	10.47	10.23	-0.24	6.35	6.20	-0.15
0.0800	11.86	11.70	-0.16	7.19	7.08	-0.11
0.1000	14.67	14.67	0.00	8.88	8.88	0.00

[a] $[KOH_o]$ = 0.1050 mole/liter; $[\underline{D}_o] + [\underline{B}_o]$ = 0.1000 mole/liter.

[b] All optical rotations in degrees.

TABLE XXV

EQUILIBRIUM CONCENTRATIONS IN THE BORATE-α-GALACTOSIDE SYSTEM

$[\underline{D}_o]$[a,b]	$[BD]$[b]	$[BD_2]$[b]	$[B]$[b]	$[D]$[b]
0.0100	0.010	0.000	0.080	0.000
0.0200	0.017	0.000	0.063	0.003
0.0300	0.022	0.000	0.048	0.008
0.0400	0.026	0.000	0.034	0.014
0.0500	0.028	0.002	0.020	0.018
0.0600	0.028	0.003	0.009	0.026
0.0700	0.025	0.004	0.001	0.037
0.0800	0.018	0.005	0.000	0.052
0.0900	0.008	0.004	0.000	0.074

[a] $[KOH_o]$ = 0.1050 mole/liter; $[\underline{B}_o] + [\underline{D}_o]$ = 0.1000 mole/liter.

[b] Mole/liter.

METHYL-α-D-MANNOPYRANOSIDE DATA

TABLE XXVI

DETERMINATION OF THE REFRACTIVE INDEX CONSTANT a

$[D_o]$, mole/liter[a]	R, scale units
0.0000	14.82
0.0200	16.26
0.0500	18.18
0.0700	19.50
0.1000	21.49
0.1000	21.47

[a] $[KOH_o]$ = 0.1050 mole/liter.

TABLE XXVII

DETERMINATION OF THE OPTICAL ROTATION CONSTANT a

$[D_o]$, mole/liter	α_{436}, °	α_{546}, °
0.0000	0.00	0.00
0.0200	1.18	0.71
0.0500	3.04	1.82
0.0700	4.21	2.54
0.1000	5.97	3.62
0.1000	5.95	3.61

[a] $[KOH_o]$ = 0.1050 mole/liter.

TABLE XXVIII

DETERMINATION OF THE REFRACTIVE INDEX CONSTANT c

$[D_o]$, mole/liter[a]	R, scale units
0.0000	16.32
0.0016	16.41
0.0032	16.54
0.0048	16.60
0.0064	16.72
0.0080	16.86

[a] $[KOH_o]$ = 0.1050 mole/liter; $[B_o]$ = 0.1000 mole/liter.

TABLE XXIX

DETERMINATION OF THE OPTICAL ROTATION CONSTANT c

$[D_o]$, mole/liter[a]	α_{436}, °	α_{546}, °
0.0000	0.00	0.00
0.0016	0.07	0.04
0.0016	0.06	0.04
0.0032	0.14	0.08
0.0048	0.18	0.11
0.0064	0.26	0.16
0.0080	0.34	0.21

[a] $[KOH_o]$ = 0.1050; $[B_o]$ = 0.1000 mole/liter.

TABLE XXX

DETERMINATION OF THE REFRACTIVE INDEX CONSTANT $\underline{d}$

$[\underline{B}_o]$, mole/liter[a]	$\underline{R}$, scale units
0.0000	21.47
0.0000	21.49
0.0030	21.50
0.0045	21.51
0.0060	21.50

[a] $[KOH_o]$ = 0.1050 mole/liter; $[\underline{D}_o]$ = 0.1000 mole/liter.

TABLE XXXI

DETERMINATION OF THE OPTICAL ROTATION CONSTANT $\underline{d}$

$[\underline{B}_o]$, mole/liter[a]	α_{436}, °	α_{546}, °
0.0000	5.97	3.62
0.0000	5.95	3.61
0.0015	5.94	3.60
0.0030	5.89	3.57
0.0030	5.96	3.61
0.0045	5.90	3.57
0.0060	5.90	3.58

[a] $[KOH_o]$ = 0.1050 mole/liter; $[\underline{D}_o]$ = 0.1000 mole/liter.

TABLE XXXII

DETERMINATION OF $\Delta\underline{R}$ VALUES

$[\underline{D}_o]$, mole/liter[a]	$\underline{R}_{exp}$, scale units	$\underline{R}_{nc}$, scale units	$\Delta\underline{R}$, scale units
0.0000	16.32	16.32	0.00
0.0300	17.81	17.86	0.05
0.0400	18.30	18.37	0.07
0.0500	18.73	18.89	0.16
0.0550	19.00	19.15	0.15
0.0600	19.22	19.40	0.18
0.0600	19.23	19.40	0.17
0.0700	19.82	19.92	0.10
0.0800	20.37	20.43	0.06

[a] $[KOH_o]$ = 0.1050 mole/liter; $[\underline{B}_o] + [\underline{D}_o]$ = 0.1000 mole/liter.

TABLE XXXIII

DETERMINATION OF $\Delta\alpha$ VALUES

$[\underline{D}_o]$, mole/liter[a]	$\alpha_{436,exp}$	$\alpha_{436,\underline{nc}}$	$\Delta\alpha_{436}$	$\alpha_{546,exp}$	$\alpha_{546,\underline{nc}}$	$\Delta\alpha_{546}$[b]
0.0000	0.00	0.00	0.00	0.00	0.00	0.00
0.0300	1.38	1.79	0.41	0.84	1.09	0.25
0.0400	1.94	2.38	0.44	1.18	1.45	0.27
0.0500	2.49	2.98	0.49	1.51	1.81	0.30
0.0550	2.79	3.28	0.49	1.71	1.99	0.28
0.0550	2.80	3.28	0.48	1.71	1.99	0.28
0.0600	3.11	3.58	0.47	1.90	2.17	0.27
0.0600	3.10	3.58	0.48	1.88	2.17	0.29
0.0667	3.55	3.98	0.43	2.16	2.41	0.25
0.0700	3.77	4.16	0.39	2.30	2.53	0.23
0.0800	4.44	4.77	0.33	2.69	2.90	0.21
0.1000	5.95	5.96	0.01	3.62	3.62	0.00
0.1000	5.97	5.96	-0.01	3.61	3.62	0.01

[a] $[KOH_o]$ = 0.1050 mole/liter; $[\underline{B}_o] + [\underline{D}_o]$ = 0.1000 mole/liter.

[b] All optical rotations in degrees.

TABLE XXXIV

EQUILIBRIUM CONCENTRATIONS IN THE BORATE-α-MANNOSIDE SYSTEM

$[\underline{D}_o]^{a,b}$	$[BD]^b$	$[BD_2]^b$	$[D]^b$	$[B]^b$
0.0100	0.007	0.003	0.000	0.080
0.0200	0.014	0.004	0.000	0.062
0.0300	0.018	0.004	0.004	0.048
0.0400	0.022	0.005	0.008	0.033
0.0500	0.023	0.005	0.017	0.022
0.0600	0.023	0.004	0.029	0.013
0.0700	0.020	0.003	0.044	0.007
0.0800	0.015	0.002	0.061	0.003
0.0900	0.009	0.002	0.077	0.000
0.1000	0.000	0.000	0.100	0.000

[a] $[KOH_o]$ = 0.1050 mole/liter; $[\underline{B}_o] + [\underline{D}_o]$ = 0.1000 mole/liter.

[b] Concentration in mole/liter.

METHYL-4-O-METHYL-α-D-MANNOPYRANOSIDE DATA

TABLE XXXV

DETERMINATION OF THE REFRACTIVE INDEX CONSTANT a

$[\underline{D}_o]$, mole/liter[a]	R, scale units
0.0000	14.82
0.0400	17.72
0.1000	21.79

[a] $[KOH_o]$ = 0.1050 mole/liter.

TABLE XXXVI

DETERMINATION OF THE OPTICAL ROTATION CONSTANT a

$[\underline{D}_o]$, mole/liter[a]	α_{436}, °	α_{546}, °
0.0000	0.00	0.00
0.0400	2.64	1.60
0.1000	6.64	4.03

[a] $[KOH_o]$ = 0.1050 mole/liter.

TABLE XXXVII

DETERMINATION OF THE REFRACTIVE INDEX CONSTANT c

$[\underline{D}_o]$, mole/liter[a]	$\underline{R}$, scale units
0.0000	16.32
0.0012	16.31
0.0012	16.31
0.0025	16.31
0.0049	16.31

[a] $[KOH_o]$ = 0.1050 mole/liter; $[\underline{B}_o]$ = 0.1000 mole/liter.

TABLE XXXVIII

DETERMINATION OF THE OPTICAL ROTATION CONSTANT c

$[\underline{D}_o]$, mole/liter[a]	α_{436}, °	α_{546}, °
0.0000	0.00	0.00
0.0012	0.08	0.05
0.0012	0.07	0.04
0.0025	0.14	0.08
0.0049	0.28	0.17

[a] $[KOH_o]$ = 0.1050 mole/liter; $[\underline{B}_o]$ = 0.1000 mole/liter.

TABLE XXXIX

DETERMINATION OF THE REFRACTIVE INDEX CONSTANT $\underline{d}$

$[\underline{B}_o]$, mole/liter[a]	$\underline{R}$, scale units
0.0000	21.79
0.0030	21.78
0.0075	21.74

[a] $[KOH_o]$ = 0.1050 mole/liter; $[\underline{D}_o]$ = 0.1000 mole/liter.

TABLE XL

DETERMINATION OF THE OPTICAL ROTATION CONSTANT $\underline{d}$

$[\underline{B}_o]$, mole/liter[a]	α_{436}, °	α_{546}, °
0.0000	6.64	4.03
0.0030	6.63	4.02
0.0075	6.50	3.95

[a] $[KOH_o]$ = 0.1050 mole/liter; $[\underline{D}_o]$ = 0.1000 mole/liter.

TABLE XLI

DETERMINATION OF THE $\Delta\underline{R}$ VALUES

$[\underline{D}_o]$, mole/liter[a]	$\underline{R}_{exp}$, scale units	$\underline{R}_{nc}$, scale units	$\Delta\underline{R}_{av}$, scale units
0.0300	17.60	17.96	0.36
	17.59		
0.0500	18.59	19.06	0.47
	18.59		
0.0600	19.09	19.61	0.52
	19.08		
0.0667	19.42	19.97	0.54
	19.44		
0.0750	19.92	20.42	0.50

[a] $[KOH_o]$ = 0.1050 mole/liter; $[\underline{B}_o] + [\underline{D}_o]$ = 0.1000 mole/liter.

TABLE XLII

DETERMINATION OF $\Delta\alpha$ VALUES

$[\underline{D}_o]$, mole/liter[a]	$\alpha_{436,exp}$	$\alpha_{436,\underline{nc}}$	$\Delta\alpha_{436}$	$\alpha_{546,exp}$	$\alpha_{546,\underline{nc}}$	$\Delta\alpha_{546}$[b]
0.0300	1.73	1.99	0.25	1.06	1.21	0.15
	1.75			1.06		
0.0500	2.99	3.22	0.35	1.82	2.02	0.20
	2.95			1.81		
0.0600	3.57	3.99	0.38	2.23	2.42	0.20
	3.64			2.21		
0.0667	3.99	4.43	0.39	2.42	2.69	0.23
	4.09			2.49		
0.0750	4.66	4.98	0.32	2.84	3.02	0.18

[a] $[KOH_o]$ = 0.1050 mole/liter; $[\underline{B}_o] + [\underline{D}_o]$ = 0.1000 mole/liter.

[b] Optical rotation values in degrees.

TABLE XLIII

EQUILIBRIUM CONCENTRATIONS IN THE BORATE-4-O-METHYL-α-MANNOSIDE SYSTEM

$[\underline{D}_o]$[a]	[BD]	$[BD_2]$	[D]	[B]
0.0100	0.009	0.000	0.001	0.081
0.0200	0.012	0.002	0.004	0.066
0.0300	0.014	0.006	0.004	0.050
0.0400	0.014	0.009	0.008	0.037
0.0500	0.012	0.013	0.012	0.025
0.0600	0.009	0.015	0.021	0.016
0.0700	0.006	0.015	0.034	0.009
0.0800	0.003	0.014	0.049	0.003
0.0900	0.000	0.009	0.072	0.001

[a] Concentration in mole/liter.

METHYL-4-O-METHYL-β-D-MANNOPYRANOSIDE DATA

TABLE XLIV

DETERMINATION OF THE REFRACTIVE INDEX CONSTANT a

$[D_o]$, mole/liter[a]	R, scale units
0.0000	14.82
0.0260	16.58
0.1000	21.87

[a] $[KOH_o]$ = 0.1050 mole/liter.

TABLE XLV

DETERMINATION OF THE OPTICAL ROTATION CONSTANT a

$[D_o]$, mole/liter[a]	α_{436}, °	α_{546}, °
0.0000	0.00	0.00
0.0260	-1.18	-0.74
0.1000	-4.58	-2.82

[a] $[KOH_o]$ = 0.1050 mole/liter.

TABLE XLVI

DETERMINATION OF THE REFRACTIVE INDEX CONSTANT c

$[D_o]$, mole/liter	R, scale units
0.0000	16.32
0.0012	16.38
0.0037	16.49
0.0062	16.60

[a] $[KOH_o]$ = 0.1050 mole/liter; $[D_o] + [B_o]$ = 0.1000 mole/liter.

TABLE XLVII

DETERMINATION OF THE OPTICAL ROTATION CONSTANT $\underline{c}$

$[\underline{D}_o]$, mole/liter[a]	α_{436}, °	α_{546}, °
0.0000	0.00	0.00
0.0012	-0.06	-0.05
0.0037	-0.16	-0.11
0.0062	-0.29	-0.19

[a] $[KOH_o]$ = 0.1050 mole/liter; $[\underline{B}_o]$ = 0.100 mole/liter.

TABLE XLVIII

DETERMINATION OF THE OPTICAL ROTATION CONSTANT $\underline{d}$

$[\underline{B}_o]$, mole/liter[a]	α_{436}, °	α_{546}, °
0.0000	-4.43	-2.73
0.0075	-4.52	-2.78

[a] $[KOH_o]$ = 0.1050 mole/liter; $[\underline{D}_o]$ = 0.0967 mole/liter.

TABLE XLIV

DETERMINATION OF THE $\Delta\underline{R}$ VALUES

$[\underline{D}_o]$, mole/liter[a]	$\underline{R}_{exp}$, scale units	$\underline{R}_{nc}$, scale units	$\Delta\underline{R}$, scale units
0.0350	17.99	18.26	0.27
0.0500	18.80	19.10	0.30

[a] $[KOH_o]$ = 0.1050 mole/liter; $[\underline{D}_o] + [\underline{B}_o] \approx 0.1000$ mole/liter.

TABLE L

DETERMINATION OF THE $\Delta\alpha$ VALUES

$[\underline{D}_o]$, mole/liter[a]	$\alpha_{436,exp}$	$\alpha_{436,\underline{nc}}$	$\Delta\alpha_{436}$	$\alpha_{546,exp}$	$\alpha_{546,\underline{nc}}$	$\Delta\alpha_{546}$[b]
0.0350	-1.68	-1.60	0.08	-1.02	-0.99	0.03
0.0430	-2.08	-1.97	0.11	-1.26	-1.21	0.05
0.0500	-2.40	-2.29	0.11	-1.47	-1.41	0.06
0.0667	-3.25	-3.06	0.19	-1.97	-1.88	0.09

[a] $[KOH_o]$ = 0.1050 mole/liter; $[\underline{D}_o] + [\underline{B}_o]$ = 0.1000 mole/liter.

[b] Optical rotation values in degrees.

TABLE LI

EQUILIBRIUM CONCENTRATIONS IN THE BORATE-METHYL-4,6-O-METHYL-β-D-MANNOPYRANOSIDE SYSTEM

$[\underline{D}_o]$[a]	[BD]	$[BD_2]$	[D]	[B]
0.0100	0.003	0.000	0.007	0.087
0.0200	0.005	0.001	0.013	0.074
0.0300	0.004	0.004	0.018	0.060
0.0400	0.003	0.007	0.023	0.050
0.0500	0.001	0.011	0.027	0.038
0.0600	0.000	0.013	0.034	0.027
0.0700	0.000	0.014	0.042	0.016
0.0800	0.000	0.013	0.054	0.007
0.0900	0.000	0.009	0.072	0.001

[a] All concentrations in mole/liter.